



**T.C.
İSTANBUL ÜNİVERSİTESİ
SAĞLIK BİLİMLERİ ENSTİTÜSÜ**

DOKTORA TEZİ

TÜRK POPÜLASYONUNDA MİKORNA'LARIN (MİR-92A, MİR-126, MİR-195, MİR-214, MİR-224 VE MİR-1247), KOLOREKTAL KANSER (CRC) TANI VE / VEYA PROGNOSTİK FAKTÖR OLARAK DEĞERLENDİRİLMESİ

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**MOLEKÜLER TIP ANABİLİM DALI
MOLEKÜLER TIP PROGRAMI**

İSTANBUL-2020

**T.C.
ISTANBUL UNIVERSITY
INSTITUTE OF HEALTH SCIENCES**

DOCTORAL THESIS

Evaluation of microRNAs (miR-92a, miR-126, miR-195, miR-214, miR-224 and miR-1247), as predictive markers of Colorectal Cancer (CRC) and/or prognostic factor in Turkish population

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MOLECULAR MEDICINE

DOCTORAL

ISTANBUL-2020

Thesis approval

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BEYAN

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Amal Srgewa

A handwritten signature in black ink, appearing to read 'Amal Srgewa', with a stylized flourish at the end.

DEDICATION

I dedicated this work to my husband and my family, who are supporting me. I also dedicated it to my university (Omar Al-Mukhtar university), which allowed me to continue my study.



ACKNOWLEDGEMENT

My most enormous gratitude is to the almighty God for giving me patience through my studies and research period. I want to express my sincere gratitude to my supervisor; Prof. Dr Sadrettin pençe, for his valuable advice, guidance, patience and immense support that has helped to bring this project to its successful end. Also, I would like to thank Doç. Dr Mustafa Duman at Kartal Hospital in Istanbul for their time and cooperation. I also have special thank go to Dr Hani Alsadoni, for his motivation, encouragement and help.



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SYMBOLS AND ABBREVIATIONS LISTS

AEBP1	: AE Binding Protein 1
ACP5	: Acid Phosphatase Type 5
AFAP	: Attenuated familial adenomatous polyposis.
AJCC	: American Joint Committee on Cancer
AKT (PKB)	: AKT/ Protein Kinase B
AMPK	: AMP-activated protein kinase
ANOVA	: Analysis of variance
AP-2 α	: Activating Protein 2 α
APC	: Adenomatous polyposis coli
APPL	: Adaptor Protein, Phosphotyrosine Interacting With PH Domain And Leucine Zipper 1
APT	: Adenine phosphoribosyltransferase
ARL2	: ADP-ribosylation factor-like protein 2
ATRA	: All-trans-retinoic acid
BCL2	: B-cell lymphoma 2
BMI	: Body mass index
BRCA	: Breast cancer gene
BubR1	: Budding uninhibited by benzimidazole 1
CACR	: Calcium-sensing receptor
CAFS	: Cancer associated fibroblasts
CARMA3	: Caspase recruitment domain and membrane-associated guanylate kinase-like domain protein 3
CASP	: Caspase
CC	: Colon cancer
CDK8	: Cyclin-dependent kinase
CDKN1A	: Cyclin-dependent kinase inhibitor 1A
CDX	: Cancer-associated fibroblast
CEA	: Carcinoembryonic antigen
ceRNAs	: Competing endogenous RNAs

CFF	: Cystic Fibrosis Foundation
CIN	: Chromosomal instability
COX-2	: Cyclooxygenase-2
cRAF	: cellular- Rapidly Accelerated Fibrosarcoma
CRC	: Colorectal Cancer
Crk	: CDPK (Calcium-dependent protein kinase)-related protein kinase
CRPC	: Castration-resistant prostate cancer
CSC	: Cancer stem cells
CT	: Computed tomography
Ct	: Threshold cycle
CTSK	: Cathepsin K
Dact2	: Dishevelled Binding Antagonist Of Beta Catenin 2
DANCR	: Differentiation Antagonizing Non-Protein Coding RNA
DCM	: Dilated cardiomyopathy
DGCR8	: DiGeorge syndrome critical region 8
DLEU2	: Deleted In Lymphocytic Leukemia 1
DM	: Diabetes Mellitus
DNA	: Deoxyribonuclease
EDTA	: Ethylenediamine tetraacetic acid
EGFL7	: Epidermal Growth Factor like domain 7
EMT	: Epithelial-mesenchymal transition
ErbB2/3	: Erythroblastic oncogene B 2 and 3
EZH2	: Enhancer of zeste homolog
FAP	: Familial adenomatous polyposis
FASN	: Fatty acid synthase
FIT	: Faecal immunochemical occult blood tests
FGF	: Fibroblast growth factors
FOBT	: Faecal guaiac-based occult blood tests
FOXD1	: Forkhead box D1
FOXM1	: Forkhead box M1

FSCN	: Fascin Actin-Bundling Protein
FXR	: Fragile X-related protein
GS	: Gastric Cancer
Globocan	: Global Cancer Observatory
GSK3b	: Glycogen synthase kinase 3 beta
HCC	: Hepatocellular Carcinoma
HMGA1	: High Mobility Group AT-Hook 1
HNPCC	: Hereditary non-polyposis colorectal cancer
HOXA11	: Homeobox A11
IARC	: The International Agency for Research on Cancer
IBD	: Inflammatory bowel disease
IGF2BP2	: Insulin Like Growth Factor 2 mRNA Binding Protein 2
JNK1	: c-Jun N-terminal kinases
KEAP1	: Kelch-like ECH-associated protein 1
KLF	: Krüppel-like factor
K-ras	: Kirsten rat sarcoma
linc-MD1	: Long Intergenic Non-Protein Coding RNA, Muscle Differentiation 1
LOH	: Loss of heterozygosity
LUAD	: Lung adenocarcinoma
MALT	: Mucosa-associated lymphoid tissue
MAP	: A mitogen-activated protein
MAPK	: A mitogen-activated protein kinase
MEK	: MAPK/ERK kinase
MiRBase	: Micro-RNA base
MiRGeneDB	: MicroRNA gene database
MiRNA	: Micro ribonucleic acid
MMR	: Mismatch repair
mRNA	: Messenger ribonucleic acid
MSI	: Microsatellite instability
MSH3	: Microsatellite instability-high

mTOR	: Mammalian TORC1
MYCBP2	: MYC Binding Protein 2)
NAP	: <i>NTHL</i> -associated polyposis
Nectl-2	: Nectin-like molecules
NF	: Nuclear factor
NFATc1	: Nuclear factor of activated T-cells, cytoplasmic 1
NFκB	: Nuclear factor kappa light chain enhancer of activated B cells
NRAS	: Neuroblastoma RAS
NRF	: Nuclear respiratory factor
NRP	: Neuropilin
NSAIDs	:Non-steriodal anti-inflammatory drugs
NSLC	: Non-small cell lung cancer
NTHL	: Nth Like DNA Glycosylase 1
OS	: Overall Survival
OSCC	: Oral squamous cell carcinoma
PC	: Prostate Cancer
PCMTD Containing 1	:Protein-L-Isoaspartate (D-Aspartate) O-Methyltransferase Domain
PDLIM2	: PDZ And LIM Domain 2
p-ERK	: PKR-like endoplasmic reticulum kinase
PI3KCA	: Phosphatidylinositol 3-kinase catalytic alpha polypeptide
PI3K	: Phosphatidylinositol 3-kinase
PI3KCA	:Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
PIM1	: Pim-1 Proto-Oncogene
Piwi	: P-element-induced wimpy testis
PHLDA2	: Pleckstrin Homology Like Domain Family A Member 2
PLR	: Pinoresinol-lariciresinol reductase
PRMT	: Protein arginine methyltransferases
POLE	: Polymerase epsilon
POLD1	: Polymerase Delta 1

PTEN	: Phosphatase and tensin homolog
PSA	: Prostate specific antigen
qRT-PCR	: Real-time quantitative polymerase chain reaction
RAB	: Ras related protein 3
RAF	: Rapidly Accelerated Fibrosarcoma
RAS	: Rat Sarcoma
RFWD2	: Ring finger and WD domain 2
RNA	: Ribonucleic acid
RNAi	: Ribonucleic acid interference
SMAD4	: Mothers against decapentaplegic homolog 4
SNHG6	: Small Nucleolar RNA Host Gene 6
SOX9	: SRY-Box Transcription Factor 9
STMN1	: Stathmin 1
TCF	: T-cell factor
TFAP2A Alpha	: Transcription Factor AP-2 alpha (Activating Enhancer Binding Protein 2 Alpha
TGM2	: Transglutaminase 2 gene
TNBC	: Triple-negative breast cancer
TNF	: Tumour necrosis factor
TNFAIP1	: TNF Alpha Induced Protein 1
TNM	: Tumor, Node, Metastasis
TPD52	: Tumor Protein D52
UC	: Ulcerative Colitis
UQCRC1	: Ubiquinol-cytochrome c reductase core protein 1
VCAM	: Vascular cell adhesion protein 1
VEGF	: Vascular endothelial growth factor
WAF-1	: Wild-type activating fragment-1
WNT	: Wingless-related integration site
YAP	: Yes-Associated Protein

ÖZET

Srgewa, A.F. (2020). Evaluation of microRNAs (miR-92a, miR-126, miR-195, miR-214, miR-224, and miR1247) as predictive markers of colorectal cancer (CRC) and/or prognostic factor among the Turkish population. İstanbul Üniversitesi Sağlık Bilimleri Enstitüsü, Moleküler Tıp ABD. Doktora Tezi. İstanbul.

Kolorektal Kanser (Kalın Bağırsak Kanseri), multifaktöriyel bir hastalık olup, ölümcül kanserlerden biridir. Geç tanı konan hastalar için kötü bir prognoz vardır. Patogenezin moleküler düzeyde tam olarak anlaşılması, CRC'nin saptanması ve tedavisi ile ilgili önemli sonuçlara yol açabilir. Ayrıca, erken non-invaziv tanısal biyobelirteçlerin kurulması, hastalık tedavisinin ve genel survi seyrine yardımcı olacaktır.

MikroRNA'lar, kanserin ilerlemesinde kritik unsurlar olup kanda saptanabilen aday biyobelirteçlerdir. miR-195, miR-92a, miR-126, miR-1247, miR-214 ve miR-224 seviyelerini CRC evreleriyle ilişkilendirmek ve Türk popülasyonundaki hasta ve kontrol grubundan örnekleri karşılaştırmak için altı miRNA'nın dolaşım ifadenme seviyesini inceledik. 40 Kolorektal kanserli ve 40 kontrol altındaki bireylerin miRNAlarındaki ifade seviyelerinin istatistiksel analizi için qRT-PCR uygulayıp, tek yönlü ANOVA ve Pearson korelasyon testi kullandık.

Çalışmamızta, miRNA'ların (miR-195, miR-224, miR-126-3p ve miR-1247) kat değişimine sahip kontrol grubuna kıyasla hastanın serumunda önemli ölçüde downregüle olduğu, (sırasıyla -18, -6.4, -9 ve -11) 0.05'ten küçük bir p değeri ile miRNA'lar (miR-214 ve miR-92a) kanserde upregüle edilirken, hastalar ve kontrol vakaları arasında düzenli olarak 6.6 ve 35 kat değişimi vardır. İlginç bir şekilde, farklı kanser evreleri arasında ekspresyon seviyesinde önemli bir fark olmasına rağmen miRNA'ların sigara içme, yaş ve cinsiyete göre ekspresyon düzeyinde önemli bir fark yoktur. Fakat miR-1247 seviyesi, sigara içen grupta daha yüksekti. Sonuç, kolorektal kanserde bu miRNA'yı biyobelirteç olarak kullanma olasılığının olduğunu gösterir.

Anahtar Kelimeler: Kanser, kolorektal, Mirna, miR-195, miR-92a, miR-126, miR-1247, miR-214, miR-224, biyobelirteç.

Bu çalışma, İstanbul Üniversitesi Bilimsel Araştırma Projeleri Birimi tarafından desteklenmiştir. Proje No: 31994

ABSTRACT

Srgewa, A.F. (2020). Evaluation of microRNAs: miR-92a, miR-126, miR-195, miR-214, miR-224, and miR-1247 as predictive markers of colorectal cancer (CRC) and/or prognostic factor among the Turkish population. Istanbul University Institute of Health Science, molecular medicine department. PhD Dissertation. Istanbul.

Colorectal Cancer (CRC) is one of deadly cancer; It is a multifactorial disease. There is a poor prognosis for patients who diagnosed late. Complete understanding of the pathogenesis at the molecular level could have significant implications concerning the detection and treatment of CRC. Moreover, establishing early non-invasive diagnostic biomarkers will aid the course of the disease treatment and overall survival.

MicroRNAs are critical elements in the progression of cancer and are candidate biomarkers detectable in blood. We studied the expression level of circulating level of six miRNA, namely; miR-195, miR-92a, miR-126, miR-1247, miR-214, and miR-224, to correlate their level with CRC stages and compare the samples from patients and control group among the Turkish population. We performed qRT-PCR, to detect the expression level of miRNAs in 40 patients have colorectal cancer and 40 control individuals, for statical analysis, we used one-way ANOVA and Pearson correlation test.

Our study revealed that miRNAs (miR-195, miR-224, miR-126-3p and miR-1247) are significantly downregulated in patient's serum compared with the control group with a fold change (-18, -6.4, -9 and -11, respectively), with a p-value less than 0.05. while miRNAs (miR-214 and miR-92a) are upregulated in cancer, having 6.6 and 35 fold-change orderly between the patients and control cases. Interestingly, there is a significant difference in expression level among different cancer stages. There is an insignificant difference in the expression level of miRNAs based on smoking, age and gender. However, mir-1247 was higher among the smoker group. The result indicates the possibility of using those miRNA as biomarkers in colorectal cancer.

Key Words: Cancer, colorectal, Mirna, miR-195, miR-92a, miR-126, miR-1247, miR-214, miR-224, biomarker. Key Words: Cancer, colorectal, Mirna, miR-195, miR-92a, miR-126, miR-1247, miR-214, miR-224, biomarker.

The Research Fund of Istanbul University supported the present work. Project No. 31994.

1. INTRODUCTION AND AIMS

1.1. Introduction

Colorectal Cancer is a deadly illness throughout the world. Globally, this cancer considers a significant public health problem, with an about 1.84 million newly diagnosed patients and over 880,792 mortality, with an approximate 9.2% of all deaths caused by cancer (IARC, 2019). There has been accumulating evidence of the incidence, and the distribution patterns of colorectal cancer are changing. Colorectal cancer has multifactorial aetiology with genetic and environmental impacts; the higher risk of Colon and rectal Cancer development is for both IBD and family history of CRC; raised risks were also related to higher body mass index (BMI), smoking of tobacco, and red meat and alcohol intake (Levine et al., 2008). Currently available non-invasive screening practices, such as “FOBT” and “FIT”, have succeeded to some extent in declining the mortality rate (Whitlock et al., 2008). Nevertheless, large-scale investigative researches have remarked the fault of these tests as a result of low precision, especially in regards to the discovery of the predisposing neoplastic lesion, those should receive the chief concentration for any cancer-screening programs (Ahlquist et al., 2008).

MicroRNAs are a kind of short non-protein coding regulatory Ribonucleic Acids; their length is 22-nucleotides in approximate, act a vital task in the gene via attaching to the 3'-UTR of mRNAs who are coding of protein. A distinct miRNA might govern several aimed messenger-RNAs; microRNAs are divined to control about Sixty % of the genes in the human (Kozomara & Griffiths, 2013). Classification and lexicon of miRNAs correspondences to development in the technology field, also the installation of the deep sequencing have implemented means for discovery and exploration of the regulatory Ribonucleicacid classes (Calin et al., 2000). The earliest evidence connecting microRNAs by malignancies in 2002, was stated by the laboratory of Croce, expressing, the deletion of 13q14 limit the expression of the “miR-15a/16-1” members, set among the DLEU2 gene, leading to CLL (Whitlock et al., 2008). Particular miRNAs expression has risen and behaved as oncogenes in one kind of cancer, while some are decreased and perform as an inhibitor gene of the tumour in a different neoplasm sample. This diversity has been

recognised for several miRNAs such as “let-7, miR-15a,miR-16/1, miR-17-92 group, miR-26, miR-29, miR-125a and miR-125b” (Cekaite et al., 2016; Zeitels et al., 2014).



Around 450 individual microRNAs were linked with CRC; the most commonly proclaimed are miR (21, 145 and 143), then come miR-(31, 34a, 200c, 20a and 92a). Exploratory studies have confirmed that in CRC a sum of 530 miRNA-mRNA couples is stated, 200 different miRNAs and 347 specific targets. Serum level of miR-34a is found to be diminished in the colorectal tumour (Nugent et al., 2012); however, in another one was elevated (Aherne et al., 2015). MiR-214 in colon tissues reduces levels of PDLIM2 of the cells by the interplays with the actin cytoskeleton through the PDZ domain. and PTEN (tumour suppressor), which enhances AKT phosphorylation, and stimulates NF κ B. The action of this loop associates with the activity of the disease in patients with Ulcerative Colitis and the progression to CRC (Polytarchou et al., 2015). Moreover, Stathmin 1 was determined to be a direct and utilitarian affector of miR-1247; The notable rise in the expression of STMN1 was identified in the tissue of CRC tissue (Zhang et al., 2016). Research's results affirmed that miR-92a has a function in the increase of EMT and the regulation of cell development, migration and invasion by the abolition of "PTEN" expression (Zhang et al., 2014). It was claimed that miR-195 is at a reduced level in the tumour tissues. Furthermore, the increase in the level of miR-195 in colorectal cancer cell lineage might diminish cell living, improve programmed cell death and repress the tumour development capacity (Liu et al., 2010). Increased the level of miR-126 has already been linked with risen VEGF-A interceded signalling in endothelial cells and greater vascular integrity (Hansen et al., 2012). In regards to cancer metastasis, MiR-224 advances CRC spread, partly by the control of "SMAD4".

MiRNAs are candidate biomarkers and key players in cancer progression with demonstrated changes of expression in different cancers. This study, therefore, set out to investigate miRNA serum level in CRC, using a small RNA sequencing approach using blood samples from CRC patients and control. The blood samples are obtained from CRC patients before receiving any treatment; the second sample is to be obtained after receiving a treatment regime for at least six months if the patients accept.

1.2. Hypothesis

1. There is no difference in the serum level of the selected miRNA between colorectal cancer samples and the control.
2. There are significant differences in serum miRNAs level between the patients and control.
3. There is a noticeable variation in the expression level of miRNA between different stages.
4. There is variation among miRNA expression level in CRC.

1.3. Project plan

Total samples number is 120 samples divided as; 40 healthy volunteers as control; 40 from the patients before receiving any therapeutic approach; and lastly if available 40 from the same patients after getting therapy. The criteria for selecting those patients that they should be newly diagnosed, no metastasis, no other cancer, and not received any treatment. The support was received from BAP; the time table of this project is one year from 4/2019 to 4/2020 as the patients should undergo therapeutic approach after taking the first sample from them.

1.4. Aims

This study's purpose is to verify the value of detecting the serum expression level of micro RNAs for the diagnosis and prognosis in the Turkish population. That could help in early diagnosis of the CRC cancer as well as be following the prognosis during treatment.

The specific objectives of this work are:

1. To determine the serum levels of miR-92a, miR-126, miR-195, miR-214, miR-224 and miR1247 of colorectal patients.
2. To estimate the differences in MiRNAs level in the serum between cancer patients and control.

3. Compare the results of treated patients with normal if the patients accept.
4. Correlate the effect of age, gender, smoking and another health status with the expression level of miRNAs.

1.5. Structure of the dissertation

This work is organised into five main chapters to cover the subject in its theoretical and practical parts. The first two are focused on the literature review related to colorectal cancer and micro-RNA, information and results of previous experiments are included. The chapters from three to five are focused on the design of the methodology, the investigation results and final findings. Thus, the chapters of this thesis are designed as the following:

Chapter one includes the introduction: an overview of the subject of the research and an identification of the research aim, objectives and structure. Chapter two concerning of Literature review: a general review about cancer and colorectal cancer, including definitions, causes, symptoms, morbidity, mortality and risk factors. Micro-RNA and a specific review on the biogenesis and functions. Also, it is concerned with the role of miRNAs is reviewed, as well as specific studies for the selected miRNAs that are studied in this research. Chapter three includes the materials and methodology: the chapter provides full information on the material that was used to conduct the experiment, in addition to the protocols, their variations and analysis methods that were used specifically for this study. Chapter four includes the results: an analysis of the results and calculations used in the research for the quantification of the six miRNAs, and the statistical analysis of the results. The last chapter concerns of discussion and conclusions: the final results of the research are summarised and discussed with the relevant literature. Suggestions and recommendations are provided for each miRNA to serve the diagnostic purpose of colorectal cancer.

2. LITERATURE REVIEW

2.1. Anatomical and microscopic features of the large intestine

The alimentary tract is ended by large intestine; it is starting with the terminal ileum and ends at the anus. In adults, the colon is about 1.5 meters long tubular structure and divides into the cecum with the appendix, the ascending, transverse, and descending colon, and the sigmoid colon, orderly. The rectum is about 12 cm long extends from the peritoneal reflexion and adjusts to the curvature of the sacral structure to the anus (Scanlon & Sander, 2014).

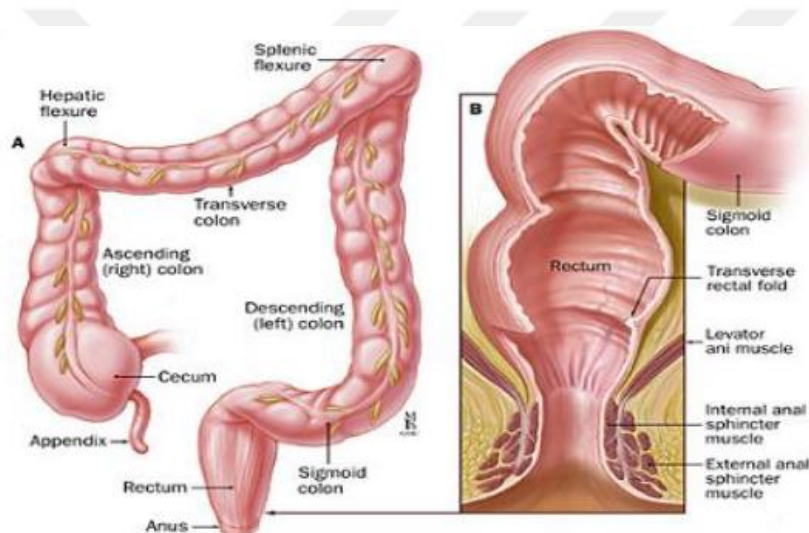


Figure 2.1: Normal colon and rectum anatomy

A. The anatomy of the colon B, Anatomy of the rectum. adopted from normal anatomy of the colon and rectum. (2013). Irritable Bowel Syndrome (IBS): Introduction

The histological features of the colon illustrate four histological patterns; the mucosa, the submucosa, the muscularis externa and lastly the serosa (Yeatman T.J., 2001). The mucosal cells are mostly where the earliest genetic changes happen as a result of their continuous cell division to replace the shed cells from the wall of the intestine towards its lumen. The mucosa of the rectum has similarity with colon's mucosa, except its goblet cells are more exceeding (Araki et al., 1996). The blood and lymphatic vessels and nerve fibre's endings are contained in the second layer submucosa. This layer is vital in the process of cancer development because if a neoplasm penetrated into this area of

the intestinal layer, it easily enters the blood and lymph, spreading within the body, it is shown in figure (2-1). (Junqueira & Mescher, 2013).

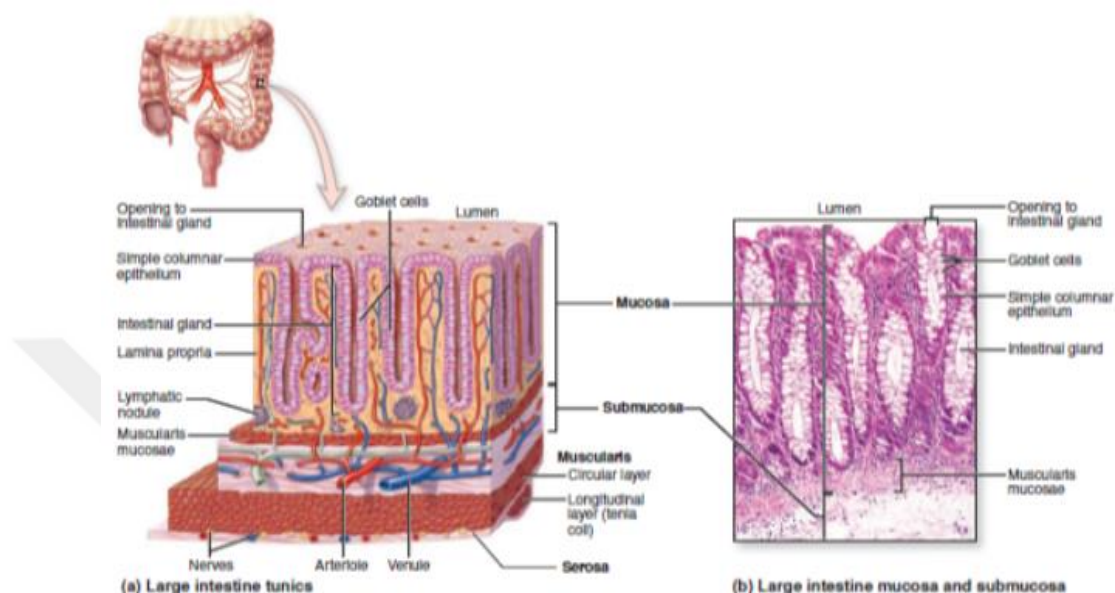


Figure 2-1: Histology of the large intestine tissue

- a) The diagram shows the wall of the large intestine composing of the four patterned layers. Well-vascularized is submucosa. The muscularis has a patterned inner circular layer, and the outer longitudinal muscle is only present in three equally spaced bands, the teniae coli
- b) The mucosa is occupied mostly by tubular intestinal glands extending as deep as the muscularis mucosae and by lamina propria rich in MALT

2.2. Colorectal Cancer

It is further pointed to a tumour of the colon and rectum. It is among the significant reasons of malignancy-related mortality universally (Bray et al., 2018). Worldwide Cancer Statistics in 2018, declared that CRC is the second and third prevalent malignancy in women in men respectively, including more than 1.2 million newly diagnosed patients and 608,700 mortality measured to have developed. The rate of occurrence and death is more distinguished in men than in women (Heer, P.D, 2007). CRC is described as a cancerous mass in the large alimentary tract; it could be regionally, or distantly spread. It extends from the caecum to the sigmoid, and from the recto-sigmoid to the anus for the cancer of the rectum (Heer, P.D, 2007).

2.2.1. Histopathology

Adenocarcinomas form the vast majority of colorectal cancers; approximately 90% in most cases; the cancer is followed adenomas or adenomatous polyps. A small percentage of adenoma, about 10% will progress into cancer within ten years (Klimstra, Arnold & Capella, 2010). Through Histological examination, three kinds of adenomas have been determined; according to the occurrence; the majority are tubular constitutes about (75%–90%), then tubule-villous and the least of adenomas are villous adenomas (3%–10%). Several criteria determine the progression of adenomas to cancer: there is a higher probability of arising cancer from adenomas if it is a villous adenoma, with a diameter larger than 1 cm, multiplicity, and dysplasia degree, the previously mentioned characteristics correlate with each other (Schottenfeld & Fraumeni, 2006).

The most frequently diagnosed colorectal polyps are hyperplastic polyps; it has been widely considered as a benign growth that rarely progresses to cancer. However, in some hyperplastic polyps, different mutations including K-ras mutations have been detected, leading to the serrated adenoma (Groff & Ahnen, 2008), which combines the serrated of a hyperplastic polyp morphological appearance with the dysplastic features of an adenoma. The data is suggesting that serrated adenomas could be precursors of colorectal cancers. Figure (2-2) shows The three primary histologic colorectal adenocarcinoma subtypes (Julien et al., 2012).

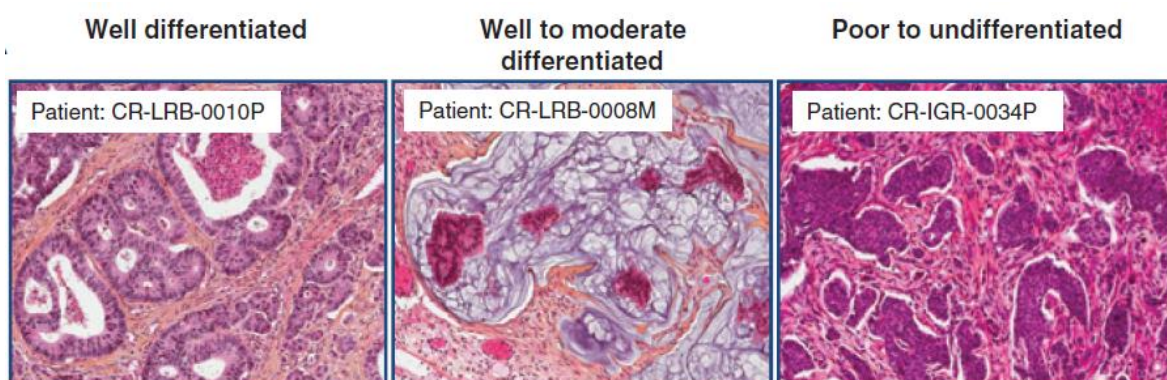


Figure 2-2: The tumour phenotype, histopathologic diversity, and stroma characterisation of the colorectal tumour collection

The three primary histologic colorectal adenocarcinoma subtypes Original magnification: $\times 200$

2.2.2. Epidemiology

Colorectal carcinoma represents the third commonly tumour, and it is among principal cancer-causing deaths in the world. There is a variation by 23-fold worldwide CRC rates between low-incidence areas in Asia/ Africa and Western areas of Europe, Australia, New Zealand, and North America. High CRC frequency and fatality rates were noticed in the further developed populations. However, despite the incidence rates in France, Italy, and England was stable or slightly increasing, and in the USA was decreasing, in the prior decades, there was an exceptional increase in CRC incidence in less-developed areas such as Eastern European countries, certain Asian countries, and several South American countries (Center & Ward, 2009). The World incidence and mortality rate in 2018 within both sexes, according to Globocan, demonstrates that CRC represents the third and the second position among cancers incidence and mortality rate, respectively as in fig 2-3 and 2-4 (Globocan, 2018). IARC (Globocan, 2018) stated that the estimated age-standardised incidence rates among females and males illustrate that CRC is cancer comes after breast cancer among women (Figure 2-5) and the third among males after cancer of lung and cancer of the prostate (Figure 2-6).

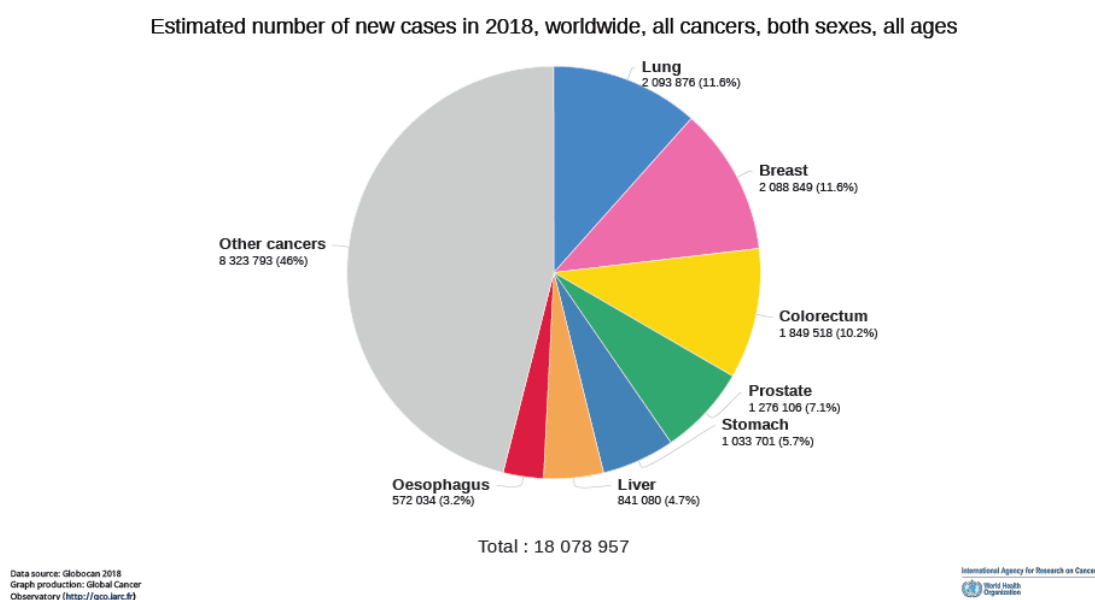


Figure 2-3: Estimated incidence in 2018, worldwide, all cancer, both sexes, and all ages

Estimated number of deaths in 2018, worldwide, all cancers, both sexes, all ages

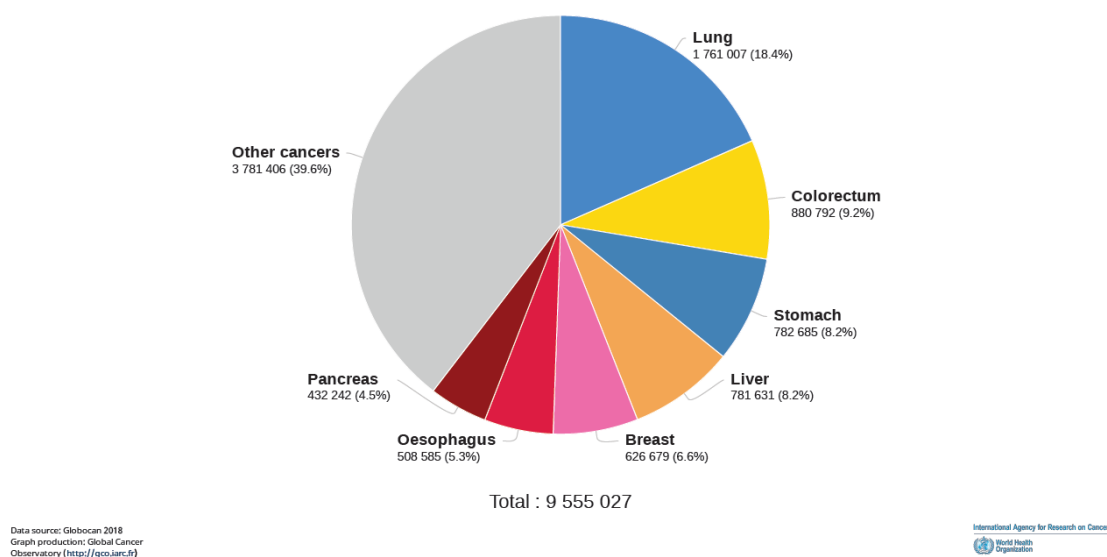


Figure 2-4: Estimated number of death in 2018, worldwide, all cancer, both sexes, and all ages

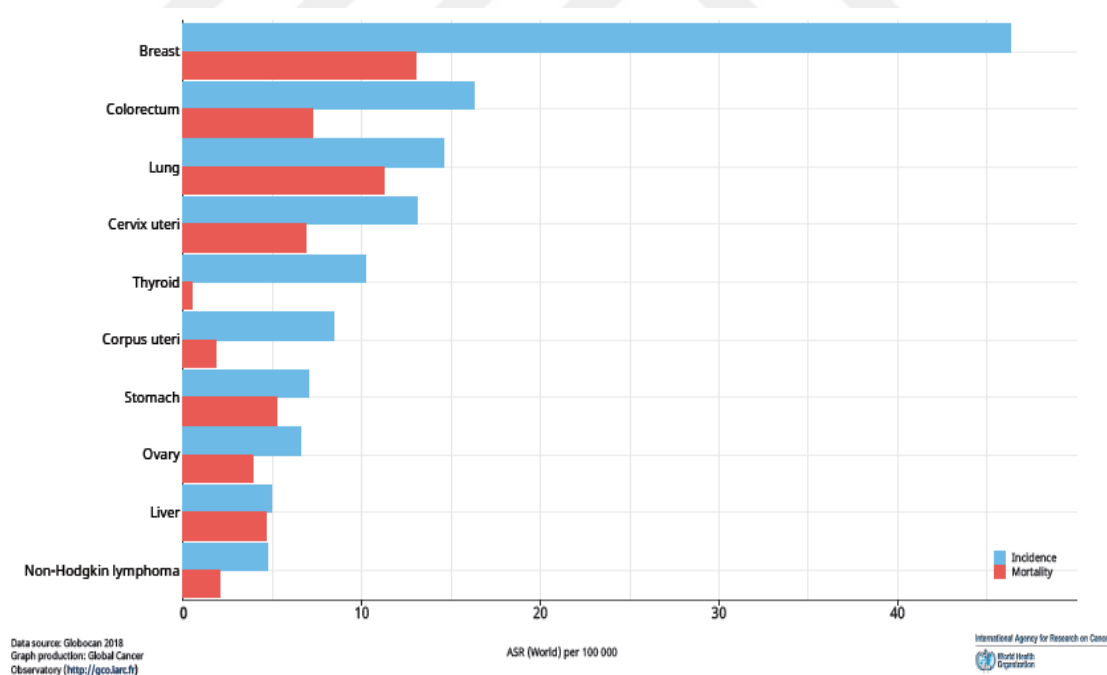


Figure 2-5: Estimated age-estimated incidence and mortality rates of cancers in 2018, worldwide, females

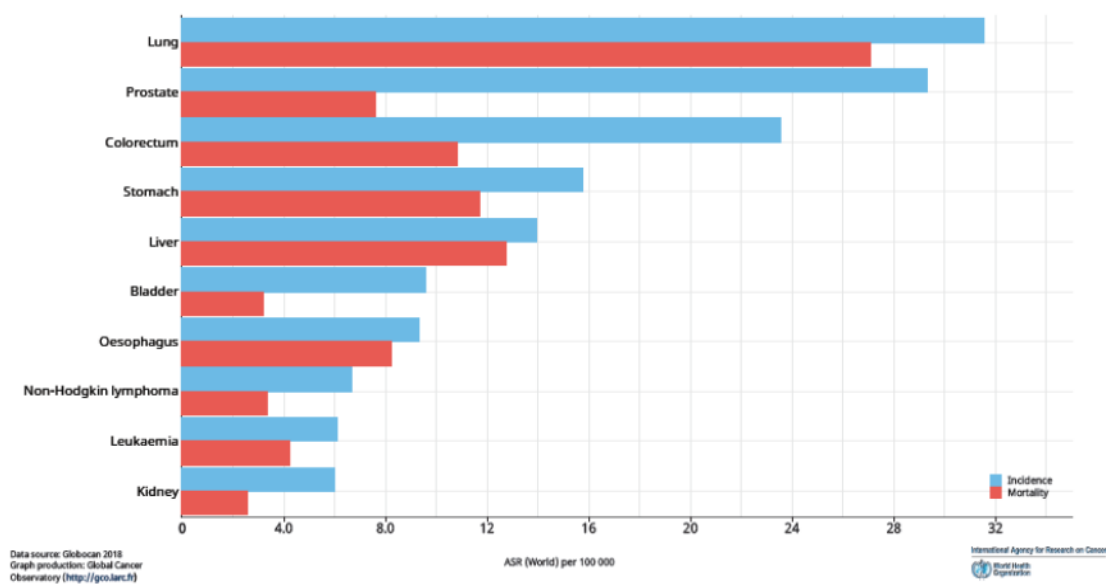


Figure 2-6: Estimated age-estimated incidence and mortality rates of cancers in 2018, worldwide, males

Growing the incidence rate of developing CRC is notices throughout the last ten years; regarding the Cancer Statistics of the “Turkish Ministry of Health” 2004-2006, the standardised frequency of cancer of colon and rectum in Turkey is 17.0 among males and 11.7 among females per 100,000 persons. Approximated total number of new CRC cases is 7,218 patients (4,102 male, 3,116 female) in Turkey, according to GLOBOCAN 2008 data (Aykan et al., 2015). In 2014, Turkey Ministry of Health Cancer Statistics demonstrated that the age-standardised incidence of CRC in Turkey is 21.0 in males, and 13.4 in females per 100,000 individuals. The estimated new patients' number in both sexes is 7,218 (3,116 female and 4,102 male) (Bray et al., 2018). Lastly, according to GLOBOCAN 2018, CRC incidence in Turkey according to the age-standardised per sex is 27.4 in males and 16.0 in females per 100,000 population. The previously mentioned data indicates that the cancer incidence is increasing in turkey throughout the ten years.

Aykan et al. (2015) showed from a multi-center registry reflection of information that cancer of the colon is commonly observed in the Marmara district, and the rectal cancer is prevalent in Central Anatolia, In the Eastern Anatolia region, upper gastrointestinal tumours are endemic. These contrasts between regions in relativistic rate

separating colon and rectal carcinoma cases might be due to circumstances related to lifestyle. As an example, in the study cohort (Aykan et al., 2015), red flesh-eating was more extra among the peoples of Marmara, Central, and Eastern Anatolia sectors. Besides, the portion of sufferers who were smoking was somewhat elevated, although insignificantly, among the patients with cancer of the rectum.

In Turkey (2018), as stated by the global cancer observatory data. The occurrence rate of CRC is still considered third cancer among males and female, after lung and prostate cancer (men), and come after breast and thyroid (in women). It is shown in (Figure 2-8 & 2-9), respectively (Globocan, 2018).

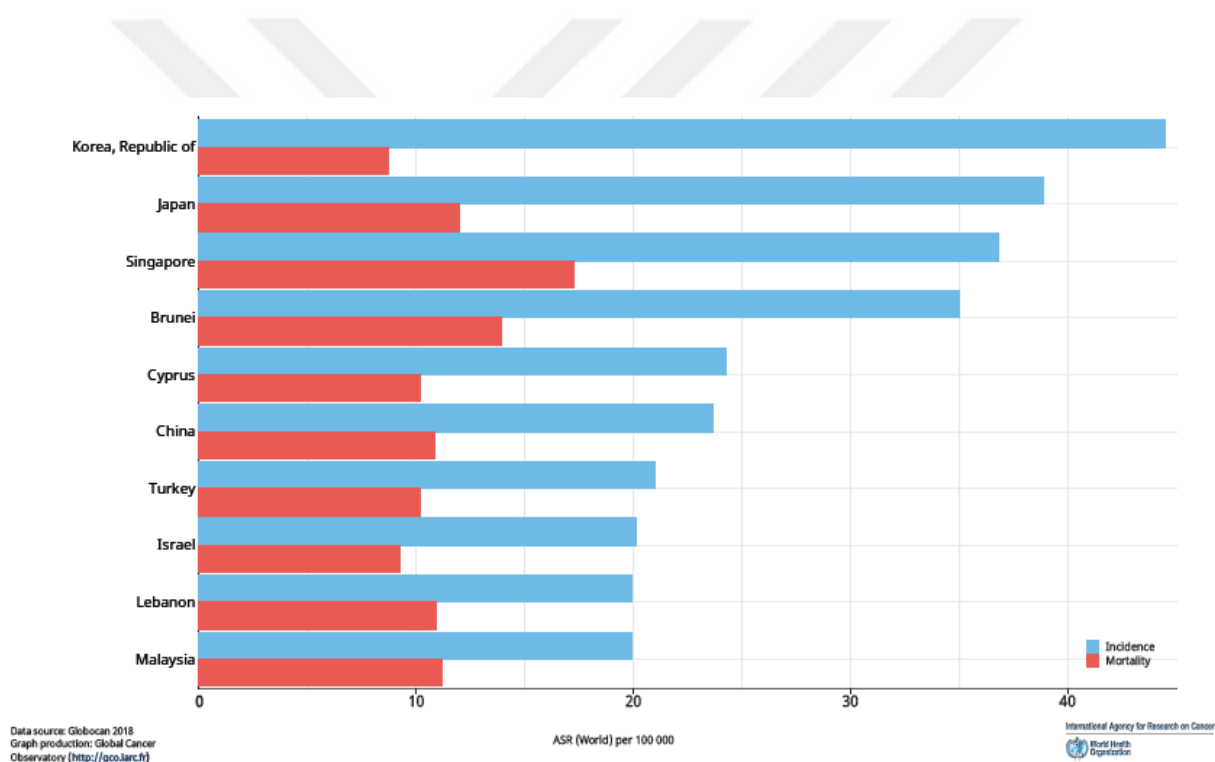


Figure 2-7: Estimated age-estimated rates Colorectal cancer in 2018, Asia, both sexes

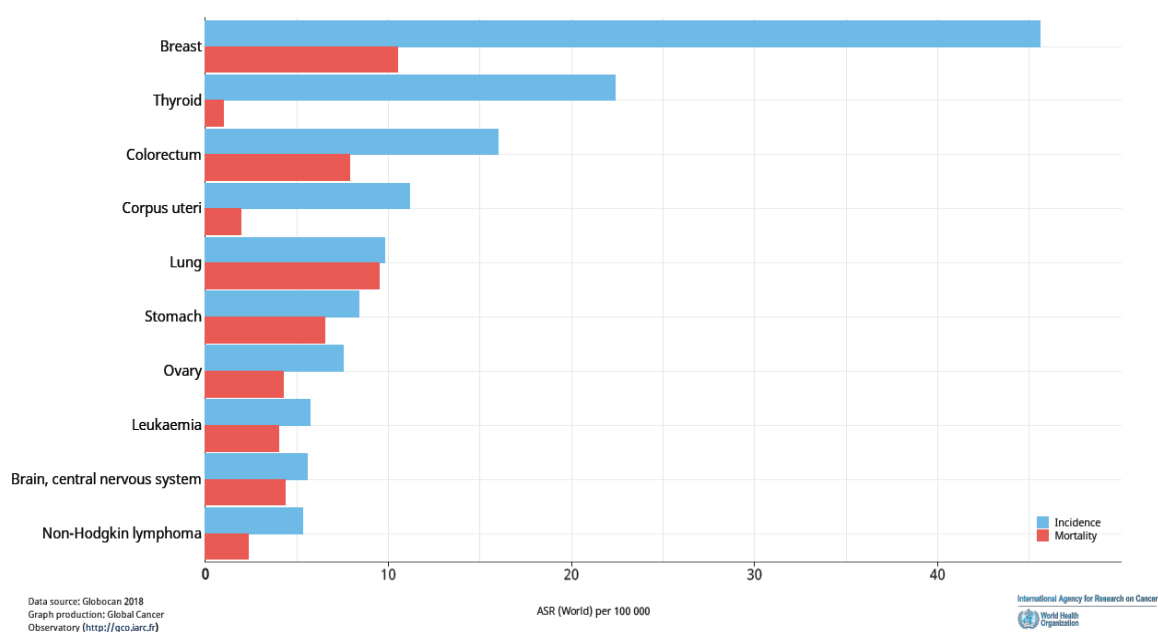


Figure 2-8: Estimated age-estimated incidence and mortality rates, cancers in 2018, Turkey, females

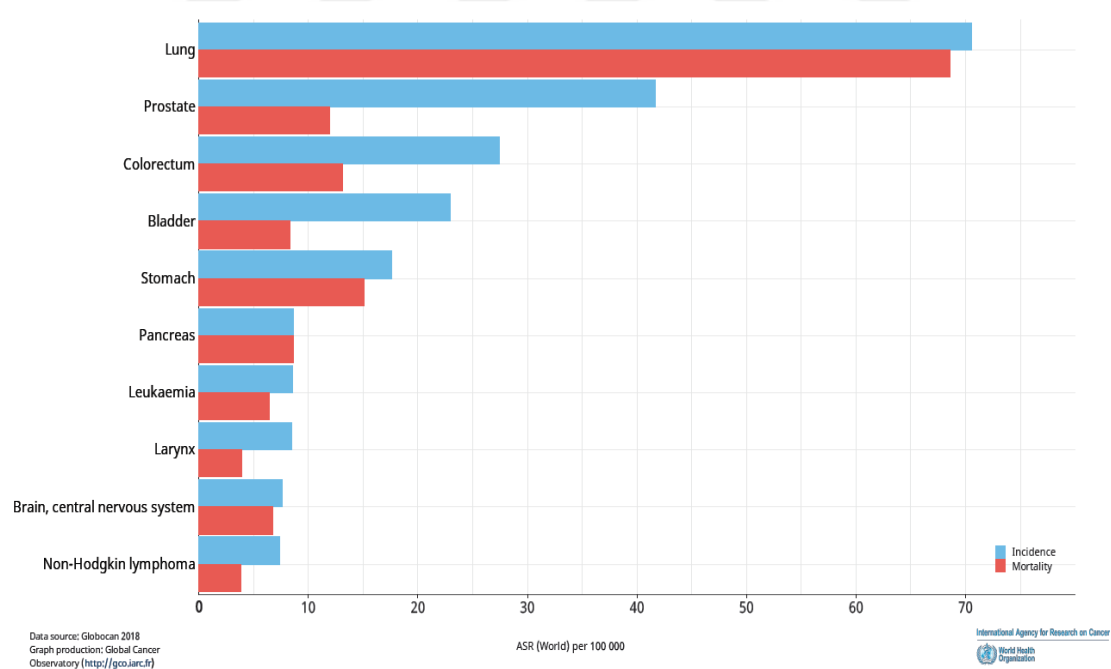


Figure 2-9: Estimated age-estimated incidence rates and mortality cancers in 2018, Turkey, males

2.2.3. Aetiology and risk factors

The aetiology of CRC is extremely complicated as it includes the contribution of environmental and genetic susceptibility factors (Wallin, U., 2011). Two significant sorts of colorectal malignancy: sporadic CRC and inherited CRC. The sporadic form represents nearly two-third of the CRC cases have no family record of the illness (Kang et al., 2011). Such incidents, the causative agents, for instance, age, lifestyle and environmental factors de novo genetic changes.

Several contributing factors increase an individual's risk to CRC, including age, the formation of polyps, IBD, style of living, genetic framework and family medicinal background (Sankaranarayanan et al., 2011). Other factors, for instance, obesity, poor diet, being physically inactive, cigarette and heavy liquor drinking, represent the cause of roughly 80% of all cases of the cancer of colon and rectum (Hagger & Boushey, 2009). Hereditary susceptibility is associated with FAP and Lynch Syndrome HNPCC estimates for 10% of all colorectal carcinoma incidents. Patients with these conditions have a higher risk of acquiring colorectal cancer reach 80% (Hagger & Boushey, 2009).

2.2.3.1. Hereditary

There are many genetic disorders mostly acquired as an autosomal-dominant pattern, causing an increase the developing cancer chances. Familial colon cancer syndromes are including; FAP and Lynch syndrome, though collectively both ailments account for only 5 % of colorectal cancer incidents, in approximate (Yurgelun et al., 2017).

FAP, with its alternatives; Gardner syndrome, AFAP and Turcot syndrome, represent fewer than 1 % of cases. Within the standard FAP, several adenomas present throughout childhood. The indications represent at a medium of 16 years old, and the cancer of colon develops in 90 % of untreated patients by the age of 45. Autosomal-recessive "MAP " disease as a result of bi-allelic germline variations in the gene of base excision repair "MUTYH". It is representing with variable phenotype, although it can present with a polyposis characteristic; ideally 500 adenomas or less. New genes related to polyposis POLE and POLD1, MSH3, and NTHL-associated polyposis [NAP] are a

member of next-generation boards with advancing practice of their characteristics (Yurgelun et al., 2017).

Hereditary nonpolyposis colorectal cancer is an autosomal-dominant syndrome that is highly prevalent than Familial Adenomatous Polyposis, and of all adenocarcinomas in the colon, it considers for around 3 %. Lynch syndrome can be surmised based on a great relative history records of colorectal cancer, endometrium, and other malignancies. Generally, cases with Lynch syndrome have a mutation in the individual allele of a Mismatch Repair gene, and another allele has somatic inactivation in the CRCs via mutation, loss of heterozygosity, or epigenetic silencing by promoter hypermethylation. CRCs which originate in subjects with Lynch syndrome is characterised through an early incipience and a predominantly of tumours at right side colon. Cancers outside the colon are widespread in Lynch syndrome, especially endometrial malignancies, that might befall in about 60 % of women carriers of the mutation. At different localities raised risk of tumour generation as in the ovary, gastric, small intestine, liver and gall bladder, brain and urinary system, and probably breast or prostate (Yurgelun et al., 2017).

In the inherited type of breast and ovarian cancer: the preponderance of cases with inherited carcinomas in breast and ovaries have mutations in one of the breast cancer susceptibility genet type 1 or 2 (BRCA1 and BRCA2). An autosomal-dominant pattern of inheritance characterises genetic breast and ovarian tumours connectable to pathogenic alternatives in "BRCA"; considerably heightened susceptibility to breast and ovarian malignancies, with an early incipience of breast tumour; and a risen occurrence of neoplasms of other organs, for instance, the fallopian tubes, prostate, men breast, and pancreas (Yurgelun et al., 2017).

2.2.3.2. Inflammatory bowel disease

A well-explained relationship linking the colonic neoplasia and Chronic UC, with the intensity, span, and the disease activity being the original indicators. Pancolitis serves a 5- to 15- times progress in risk confronted with the occurrence in the overall society, while the condition that is left side restricted is linked with almost a 3-fold risk; however,

there is no significantly increased in the risk with proctitis or proctosigmoiditis alone. A confirmation that parts of the remedies for inflammation of colon might reduce colorectal cancer predisposition and latent disorder bears less risk than the long-lasting progressive illness, maintaining a decline in the incidence of inspection (Munkholm, P., 2003).

Consistent estimation of the colorectal cancer occurrence is roughly 0.5 % per year for individuals with a span of the disease within 10 to 20 years, followed by 1 % per year after that. Several studies propose that the occurrence of ulcerative colitis and primary sclerosing cholangitis together recognises patients class with higher susceptibility. The development of pseudopolyps has been identified as an individualistic risk determinant, especially if big and complicated. Strictures should perpetually increase the doubt of cancers. The risk increase of colon cancer starts approximately a decade followed the primary determination of pancolitis, and at 15-20 years after diagnosis of left colon colitis. The developing possibility of malignancy increases with the period of the disease and with ongoing inflammation; after 40 years of disease duration, it stands as high as 30 % in subjects having pancolitis (Munkholm, P., 2003).

Even though there is extremely more limited information, it seems that pancolitis resulted with CD is connected with a proximate risk of colonic tumours as widespread UC. Guidance from practised organisations range for Crohn disorder wherever there is mild entanglement of the colon, yet most utmost recommendations suggest monitoring if 25% or more of the mucosa of the colon is affected (Munkholm, P., 2003).

2.2.3.3. Abdominopelvic radiation

Cancer survivors who underwent abdominopelvic radiation are at higher risk of following cancers of gastric and intestine, the preponderance occurring colorectal cancer. Recommendation from the Children's Oncology Group advise colonoscopy of a period of five years for childhood malignancy survivors that experienced abdominal, pelvis, spine, or total-body irradiation, with monitoring starting five years following radiation or by 30 years, whichever occurs last. Screening options include colonoscopy every five years (preferred) or multitarget stool DNA analysis every three years.

Radioactivity treatment exposure among prostate tumour patients was linked with an augmented risk of carcinoma of rectum in a couple of extensive database researches. The degree of risk is relatively equivalent to that witnessed in subjects with a family archive of adenomas of the colon. It is confusing that, if such diseases emerge from an adenoma to carcinoma progression, and if the enhanced monitoring in those sufferers might enhance tumour discovery standards and consequences. In opposition to grown-up survivors of childhood malignancy, heightened monitoring is not advised in this subsets.

2.2.3.4. Other diseases

Cancer is one of cystic fibrosis complication. Several case reports represented that there is 3-8 fold risk elevation of developing cancers of CRC among individuals with CF compared to non-CF individuals (Fink et al., 2017). Patients with cystic fibrosis have a high risk of developing CRC. In a meta-analysis, the pooled standardised incidence ratio was 10.91, and it was two- to five fold higher following lung transplantation (Hadjiliadis et al. 2018).

2.2.3.5. Age

Nevertheless, CRC affects all ages; there is an immense risk of getting the disease with increasing age. Collecting genetic issues in old tissues explain this increased susceptibility with getting older (DePinho, R. A. 2000). World cancer research (2007) reported that the probability of getting CRC increases after 40 of age, then rising sharply after the 50s. CRC which discovered in subjects more youthful than 45 years "early-onset" is comparatively uncommon, estimating for 2-8%.

2.2.3.6. Behavioural and Lifestyle factors for Colorectal Cancer

While it is well established, diet is a possible predisposing cause for several health problems, and one of them is cancer; this topic was extensively inquired. In 2001, Terry et al. claimed that there is a connection linking the decrease intake of green food and

raised liability of CRC. It is speculated that the contents of vegetables and fruits such as; C and E vitamins, fibre, folate, selenium, or phytochemical play a protective effect (Terry et al., 2001). The geographic diversity in colorectal malignancy occurrence percentage could be based on the variation in dietary fibre consumption as higher consumption of dietary fibre enhance the bulkiness of the stool and decrease the transit time of the colon (Hagger et al., 2009).

Worldwide, CRC incidence fluctuates internationally by more than ten times, with the topmost percentage in Australia, Europe, New Zealand and North America, while in Africa and Asia is the lowest (Jemal et al., 2011). The topographical variations seem to be related to inequalities in dietary and environmental factors (Center et al., 2009). On the contrary, other studies suggested that beef, pork, fat and cholesterol-rich diet might be correlated with the evolution of CRC, notably in the left side of the colon (Willett et al., 1990).

The exercise has a purpose in reducing the risk of CRC, and it is now well verified, a meta-analysis established that routine workout diminished the chance of colonic cancer by approximately 25% in males and females, as it has an effect of altering prostaglandins level and reducing stomach transportation time and enhancing the role of the immune system (Fung & Brown, 2013; Samad et al., 2007; Wolin et al., 2007).

Being over-weight is now a confirmed documented predisposing factor for CRC, Moghaddam et al., (2007) settled an analytical study by measured that people with a BMI more than 30 had a twenty percent more elevated risk of forming colorectal cancer correlated to average weight BMI individuals. Metabolic abnormalities such as hypertension, obesity, hyperinsulinemia and T2DM are linked with insulin resistance syndrome: which is as well as individuals connected to CRC (Giovannucci E, 2007). DM increases the risk of up to 30% according to the result of a meta-analysis study (Larsson & Wolk, 2006). Polycyclic hydrocarbons, nitrosamines, and Heterocyclic amine, are carcinogens present in tobacco; therefore, the long term tobacco consumption increases the possibility of developing malignancy. Studies showed that there is an elevated risk of having neoplasm and progress the colorectal tumour extension, and raise the CRC predisposition (Hagger & Boushey, 2009).

2.2.4. The symptoms of CRC

The manifestations rely on growth location, mass, the extent of CRC, includes bloody discharge from the rectum, abdominal discomfort, alternating constipation and diarrhoea, and unintentional decrease in weight. Right side carcinoma present right iliac fossa pain and mass, severe anemia, melena, weight loss, nausea, vomiting, anorexia, fainting, appendicitis. Left side carcinoma manifests by alteration of bowel habit, bleeding per rectum, weight loss, lower & left iliac fossa pain. Rectal cancer presents by bleeding per rectum, palpable mass on rectal examination, spurtial diarrhea, weight loss, tenesmus and sacral perineal pain. Emergency presentation; patient may present as an emergency case in the form of - acute intestinal obstruction- perforation result in fecal peritonitis- sever per rectal bleeding or melena- Metastasis presentation includes: jaundice, fistulae, cough (Boyle & Langman,2000).

It has been confirmed that colorectal cancer related to its main symptoms when reported to primary care (Astin et al., 2011). complains such as body mass decline and alteration in intestinal habit increased suspension of bowel tumour if associates with haemorrhage from the rectum, compared to the complaint of rectal bleeding alone. Conversely, other symptoms include loss of appetite, diarrhoea, hard stool, and perianal traits, appeared to lower the indication of colorectal cancer when accompanying the bleeding. The indications commonly deemed essential for CRC determination are bleeding from the rectum, alternation in intestinal habit, abdominal distress, decrease in weight (Adelstein et al., 2011).

2.2.5. Diagnosis of Colorectal Cancer

The diagnosis of CR Cancer relies on the pattern of complaining. The diagnosis could be incidentally during operative intervention if a subject manifests as a crisis complaining of peritonitis. Nevertheless, in the voluntary framework, colorectal cancers are typically determined by visualisation via endoscopy or through a radiology examination such as Barium enema, CT or CT colonography. Concerning the

preponderance of incidents, the endoscopic biopsy is performed to obtain histological confirmation.

2.2.5.1. Diagnostic modalities of Colorectal Cancer

Multiple outlooks of the evaluation of the CRC patients are proposed. Firstly, an established determination; secondly, the size and spreading of the growth should be confirmed; and lastly, the robustness of patients for therapy should be assessed (Rosman & Korsten, 2007). The patients who expected to have CRC must be entirely physical examined that includes a rectal inspection and palpation. Among a quite considerable fraction of subjects, the digital evaluation of the rectum reveals a firm mass, oozing on palpation. Colon endoscopy is performed to visualise the mucosa of the colon and to obtain specimens for pathological and histological investigation; Virtual colonoscopy and Magnetic Resonance colonoscopy are furthermore often practised. Regarding the carcinoma of the rectum, besides colonoscopy, clinical examination is generally applied for accurately representing modern imaging results. While, Flexible sigmoid endoscopy, screening just the lower colon, is another option to colon endoscopy and is competent in identifying the preponderance of the neoplasms of colon and rectum (Thompson et al., 2008).

2.2.5.2. Laboratory abnormalities of Colorectal Cancer

Assumed patients with colonic malignancy have to undergo regular serum investigations including a complete blood picture, glucose level measurement, electrolytes in serum and protein, investigation of biochemical indicators of hepatobiliary enzymes in serum, and the coagulation estimation. Approximately half the patients who are having a tumour of the colon are complaining of anaemia (Cappell & Goldberg, 1992).

2.2.6. Pathological stage of Colorectal Cancer

The pathological staging of the tumour mainly defines the outcome of colorectal cancer. The commonly used scheme for cancer staging is the TNM system "Tumor, Node, Metastases" and the Dukes classification. It identifies the extension of the tumour

and proper treatment. TNM staging sketched by the "American Joint Committee on Cancer (AJCC)". It has triple elements: T for the initial growth, N for the local LN and M for the disease spread, that is coupled to form grade classifications (Fleming et al., 1997). In newly diagnosed individuals have CRC, abdominal, pelvic and chest CT are employed to identify the degree of regional growth size and verify the spreading to localised lymphatic and remote metastases. The proper rectal grading framework is vital to decide on the prerequisite of neoadjuvant therapy in the malignancy (Brown, G., 2005). The following table (Table 2-1), represent the staging system of Colorectal Cancer (Weisenberg et al., 2019).

Table 2-1: The staging of colorectal cancer using the TNM system

The TNM staging of colorectal carcinoma	
Primary tumour (pT)	
• TX: primary tumour cannot be assessed. • T0: No evidence of primary tumour. • Tis: is carcinoma in situ, intramucosal carcinoma with the involvement of lamina propria with no extension through muscularis mucosae. • T1: tumour invades submucosa (through the muscularis mucosa but not into the muscularis propria. • T2: tumour with muscularis propria invasion. • T3: tumour invades throughout the muscularis propria into the pericorectal tissues • T4: ○ T4a: tumour invades through the visceral peritoneum (including gross perforation of the bowel through the tumour and continuous invasion of the tumour through areas of inflammation to the surface of the visceral peritoneum) ○ T4b: tumour directly invades or adheres to other adjacent organs or structures	
Regional lymph nodes (pN)	
• NX: Regional lymph nodes involvement is not assessed. • N0: no metastasis to the regional lymph node. • N1: 1 - 3 regional lymph nodes metastasis: ○ N1a: 1 regional lymph node involvement. ○ N1b: 2 - 3 regional lymph nodes. ○ N1c: there are no positive regional lymph nodes, but there are tumour deposits in the subserosa, mesentery or non-peritonealised pericolic or peri-rectal / mesorectal tissues	

- N2: 4 or more lymph nodes metastasis.
 - N2a: Metastasise to 4 - 6 regional lymph nodes
 - N2b: involve seven or more regional lymph nodes.

Distant metastasis (pM)

- M0: no distant metastasis by imaging.
 - M1: there is a distant metastasis
 - M1a: confined metastasis to one organ or site without metastasis to the peritoneum.
 - M1b: confirmed metastasis to 2 or more sites or organs without peritoneal metastasis
 - M1c: confirmed metastasis to the peritoneal surface alone or with other site or organ metastases.
-

In regard to “the American Joint Committee (AJC)” 2018; After combining each element of staging, the overall grade distinguished by the Roman numerical system; the stages are advancing from stage 0 to stage IVB, like the following (Table 2-2).

Table 2-2: it shows the grouping of TNM characteristics of Colorectal Cancer

Stage	Tumour	Lymph Nodes	Metastasis	Stage	Tumour	Lymph Nodes	Metastasis
Stage 0:	Tis	N0	M0	Stage IIIB:	T3 - T4a	N1 / N1c	M0
Stage I:	T1 - T2	N0	M0		T2 - T3	N2a	M0
Stage IIA:	T3	N0	M0		T1 - T2	N2b	M0
Stage IIB:	T4a	N0	M0	Stage IIIC:	T4a	N2a	M0
Stage IIC:	T4b	N0	M0		T3 - T4a	N2b	M0
Stage IIIA:	T1 - T2	N1 / N1c	M0		T4b	N1 - N2	M0
	T1	N2a	M0	Stage IVA:	any T	any N	M1a
				Stage IVB:	any T	any N	M1b

2.2.7. Survivals rate of CRC patients

The survival of subjects with CRC enormously identified by the degree of the illness at the onset of examination, the survival of 5-year rate fluctuates according to the

stage from a 90% within malignancies identified at the localised grade; 70% for provincial; to 10% for individuals with further distant spread (Jemal et al., 2004). Besides illness-free and on the whole survival period, "life quality" is essential, idiosyncratic and centred on an individual frame, including "functional, physical, emotional, and social" well-being, so it is vital outcome describing the consequence of the condition not only on the patient but on families and societies (Marventano et al., 2013).

2.2.8. Prognostic factors

The prognostic factors aid the clinicians to anticipate the disease course in cases and the most expected disease outcome, that promote medical settlement and proper therapy (Siegel et al., 2011). The prognosis of CRC depends on several factors; therefore, it widely differs among patients.

The importance of getting knowledge regarding diagnosis is for a couple of causes that affect the choice of treatment and follow up. The first reason is to determine subjects in stage I-II who are at a higher chance of redeveloping cancer; those might profit from the adjuvant medication, also to recognise subjects in stage III having a lower liability of re-occurrence, those have not to be treated with adjuvant therapy. The Second cause, to establish construction and depth of follow up plans; that is based on estimated the risk of reappearance individually in the subject to evade additional and expensive investigations (Sio, O. H, 2012).

2.2.8.1. Carcinoembryonic antigen (CEA) as a prognostic marker in colorectal cancer

Carcinoembryonic antigen is introduced for its diagnosis and prognosis uses in CRC. However, there is still conflicting data regarding its value in the prognosis in patients with diagnosed CRC. Some researches showed that individuals with a higher level of CEA preoperatively have a more unsatisfactory consequence than patients with lower concentrations (Grande et al., 2008). rise of carcinoembryonic antigen prior to operation and the extent of rising has a parallel association with heightened probability of frequency and reduced extended survival rate (Wigger et al., 1988). Moreover,

investigating groups of subjects with stage I/II, the level of carcinoembryonic antigen preoperatively is stated to be a notable determinant of prognosis (Harrison et al., 1997). In a study which followed potentially 30 curative resections, if recurrence occurs CEA may rise, with reported 64% sensitivity and 91%, specificity (Tan et al., 2009). Moreover, when the level is average preoperatively, it will increase in at least in the half of the subjects with the repetitive disorder (Grossmann et al., 2007), presenting it as beneficial in cyclic screening schedules. Nowadays, the carcinoembryonic antigen is commonly estimated postoperative as a method of identifying tumour reoccurring or screening effect of therapy (Hurwitz et al., 2004).

2.3. Pathogenesis of colorectal cancer

It encounters complicated and disparate pathogenesis which is affected by several influences, such as those related to lifestyle and diet, genetic predisposition, and the presence of diseases such as inflammatory bowel diseases (IBD) (Zie & Itzkowitz, 2008). Epidemiological studies aided in the determination of the engagement of multiple environmental and dietary circumstances, for instance, alcohol, tobacco smoking, a food rich in fat and scarce in fibre, obesity and a stationary style of living (Le Marchand et al., 1997).

While others identified some factors which have a protective effect such as, Physical activity (Slattery, M. L., 2004), extended-duration treatment with low-dose aspirin medicine (Rothwell et al., 2010), and the diet of Mediterranean region (Agnoli et al., 2013), Fearon and Vogelstein addressed that CRC pathogenesis changes according to genetic or epigenetic alterations, which follow several stages pattern as the authors theorised, these changes at the genetic and epigenetic level are straightly pledged for a precise process within the CRC causing sequence, which contributes to the beginning of the growth process of the normal epithelium or the advancing of the existed illness (Fearon & Vogelstein, 1990).

Most CRC described by diffuse loss of heterozygosis and obvious chromosomal abnormalities that are the characteristics of the chromosomal instability (CIN) pathway (Lin et al., 2003; Leary et al., 2008). DNA Mismatch Repair system and significant microsatellite instability Derangement lead to approximately around 15 percent of

Colorectal Cancer. The mismatch repair system is taking charge of the generation of the protein that identifies and straightly correct single-nucleotide mismatches at microsatellite sequences that evade the DNA polymerase proofreading system. Recently, other systems and pathways have been established to be implicated in the aetiology of CRC, which includes inflammation, unnatural DNA methylation, and further newly discovered contribution of microRNA to the carcinogenic process. CIN pathway is the accepted well-characterised and common class of the CRC routes. The means of tumorigenesis include various mitotic spindle checkpoint controllers and mutually affected protein the balance of "mitotic chromosome" (Bardi et al., 1995).

2.3.1. WNT Signaling Pathway

The mutation in adenomatous polyposis coli (APC), tumour suppressor gene, is considered. The early mutation in the process, which is implicated in both sporadic Chromosomal Instability and, when there is a germline mutation, in Familial Adenomatous Polyposis (Shih et al., 2001; Sieber et al., 2002), Familial Adenomatous Polyposis disease, is an autosomal dominant genetic syndrome distinguished by the evolution of 100s to 1000s adenomas in the colon and rectum zone during the adolescent period.

The APC is included in the APC/ β -catenin/Tcf pathway. this gene inhibition causes an elevated WNT signalling, via the inability to debase β -catenin. The cytoplasmic accumulation of " β -catenin" results in its transposition into the nucleus and triggers the TCF-targets stimulation, leading to increase proliferation, differentiation, immigration and adhesion of colorectal cells. Interestingly, individual mutations of β -catenin in 48% of tumours unaccompanied with APC mutations have been reported; there are gene mutations associated with APC/ β -catenin/Tcf pathway in Colorectal Cancer without mutations in Adenomatous polyp coli gene similarly observed in sporadic CIN neoplasms (Sparks et al., 2008).

Moreover, different WNT/APC/ β -cat pathway components could have mutations in both direct or indirect way, for instance, through stimulating " β -catenin" or Tcf.

Indirectly, the β -catenin activity might be enhanced due to the modifications in its activity regulating oncogenes in different aspects. Mutually interaction of β -Catenin with several divisions of the Notch pathway, critical controllers of the differentiation of the cells, and more lately, it has determined that it is applied in Colorectal tumourigenesis (Van et al., 2005). Researchers stated that the accumulation of active β -Catenin protein increased by Notch1 without the need for ligand-receptor stimulation (Kwon et al., 2011); it has been stated as well that the long term utilises of NSAIDs, Ibuprofen, in particular, stimulate an inhibition of Notch signalling activity which is depending on the dose, which affirms the preventive influences that have been thoroughly investigated concerning the function of Non-Steroidal Anti Inflammatory Drugs on colorectal cancer.

CDK8 amplification location is at 12q12.13 and claimed to be in about sixty percent of colorectal carcinoma patients. CDK8 kinase serves as an oncogene when its activity is increased, and its oncogenic activity appears by stimulating both β -Catenin (Firestein et al., 2008) and Notch1, which drives an enhanced transcription and cell differentiation (Fryer et al., 2004). Firestein and colleagues claimed that there is a definite correlation connecting the expression of CDK8 and β -catenin enhancement, overexpression of FASN and p53 expression, Cyclin-Dependent Kinase-8 overexpression is as well considerably correlated with a poor Colorectal Cancer prognosis (Firestein et al., 2010). Morikawa and colleagues found that overweight and physically inactive individuals are at an elevated risk of acquiring CRC with unaffected the WNT/beta-catenin pathway (Morikawa et al., 2013).

2.3.2. RAS Pathway

As mentioned above, the CIN pathway is the first event in the process of tumorigenesis; it is followed by consequence activations that stimulate new mutation and promote neoplasm progression as malignant and metastasis. The transition of benign to cancer is identified initially by a proto-oncogene K-ras gene; it encodes the GTPase protein included in the extracellular signals transfers and proliferation via mitogen-activated protein kinase. Mutation in K-ras results in a lastingly energetic state that the cells are allowed to escape apoptosis and obtain a permanent proliferation state.

Approximately ninety percent of K-ras mutation occurred at 12 & 13 codons (Malumbres & Barbacid, 2003).

The signalling pathway of Ras is additionally engaged alongside different signals essential for cancer genesis beginning. It is confirmed that more significant WNT activity correlates with higher MAPK signalling, in Colorectal Cancer samples having mutated K-ras. Moreover, intercommunication within the AMP-activated protein kinase (AMPK) and MAPK has been established (Horst et al., 2012). AMP-activated protein kinase is a sensor of the energy homeostasis in the cells, via the repression of the mTOR signalling and stimulation of the CDKN1A (p21) and p53 pathway and performs a task in the control of cell propagation and germination. However, Enhanced display of phosphorylated-AMPK is allied by a favourable outcome within p-ERK-activated Colorectal Cancer subjects (Baba et al., 2010).

2.3.3. The p53 System

Chromosome 17p is the position of the P53 gene; the alteration is an example of main events in CR carcinogenesis, which activate proliferation via cell cycle control and apoptosis loss. A study in 2005, demonstrated that p53 affected the transcription and expression of BubR1; patients who have 17p LOH, the activity BubR1 is remarkably reduced (Oikawa et al., 2005).

El-Deiry et al., (1993) determined a wild-type p53-activated fragment 1, which is instantly controlled by p53, that represses tumour growth in the pathway of p53. If p53 is altered, the protecting function of WAF-1 is not expressed. Also, p53 serves as a regulator of energy balance as a consequence of the AMPK pathway activation. This role is studied further by Morikawa et al., and reported that within the group of normal-weight subjects, p53 was correlated with lowered patient survival in contrast with obesity effect on Colorectal Cancer cases death was observed in p53 mutated individuals (Morikawa et al., 2012).

Up-regulation of the CDKN1A by P53 is through the AMPK pathway, that is contributed in the control of the cellular cycle. Ogino et al., (2009) noticed that p21 lack of function in 79% of Cancer in colon and rectum cases and it is linked with the expression

of p53. They exhibited a positive association between the loss of p21 and Colorectal cancer survival with elder patient, in particular for individual elder than sixty years while the obesity effect in Colorectal Carcinoma is not recorded in p21 lack Colorectal Cancer.

CDKN1B is in addition connected with p53, is implicated in the advancing control into the cell cycle (S phase), and its degeneration is correlated with the progress of CRC (Loda et al., 1997). P53 as well collaborate with Cyclooxygenase-2 that has a function in increasing inflammatory response and cell propagation in colorectal cancer (Swamy et al., 2003). Intriguingly, increased cancer-specific mortality was found to be associated with COX-2-positive tumours with despite p53 state, suggesting that Cyclooxygenase-2 could be an objective predictive element of colorectal cancers (Ogino et al., 2008).

2.3.4. Other Pathways

In 65.4%, loss of heterogeneity of chromosome 18q occurs with the p53 accompanying molecular modification, that region Smad2, Smad4 and DCC genes are discovered (Lanza et al., 1998). LOH of 18q is strongly linked to an unfavourable outcome in colonic malignancy, particularly with distinguished metastatic liability (Kern et al., 1998). The research revealed that there was an advantageous clinical consequence is detrimental Colorectal Cancer 18q allelic loss. They displayed that the five years living rate with illness-free in these patients was 96% (Lanza et al., 1998).

The simultaneous change that often transpires along with the mutations of the APC gene is found in the phosphoinositide-3 kinase) signalling pathway (Deming et al., 2014), that causes the stimulation of various nuclear transcriptional factors via kinase cascade. PI3KCA gene mutations induce cell advancement and FASN generation through the AKT pathway (Samuels & Velculescu, 2004). Mutations of PI3KCA and communicate with a prime controller of cell growth and metabolism, mTOR, and K-ras. They indicated that the concomitant residence of APC and PI3KCA gene anomalies in animal versions is correlated with enhanced tumour proliferation and extent, and further intrusive behaviour (Deming et al., 2014). Lastly, Liao et al., (2012) observed that the usage of aspirin for long term prolonged the survival in patients with mutated-PIK3CA

CRC, probably by stimulating apoptosis through PIK3CA hindrance. It was theorised that long interspersed nucleotide element-1 (LINE-1) extreme hypo-methylation is correlated with the chromosomal instability pathway. In LINE-1 Retrotransposon, L1 represents around 17% of the individual genome, eases the genomic and instability of chromosome through global DNA hypomethylation and contributes to the expression of non-coding RNA (Cordaux & Batzer, 2009). Ogino et al., (2008) demonstrated a reverse association between the MSI pathway and LINE-1 hypo-methylation, it also confirmed that the presence of a direct connection between LINE-1 hypomethylation and chromosomal instability (Baba et al., 2010).

2.4. Molecular Markers

The following table (table 2-3), illustrates the makers that reviewed by Colussi et al., (2013) in their study, so it provides the choice of possibly with using multiple molecular markers, to predict the behaviour of the disease in newly cancer-diagnosed patients. Hopefully, it will help to define the therapeutic strategies.

Table 2-3: The molecular markers and implications for disease behaviour

Gene	Effect on disease
CDK8 overexpression	Poor prognosis
K-ras cod. 12 mutation	Metastatic disease; poor prognosis; increased cancer-specific mortality p-AMPK Better survival among p-ERK positive
p53 expression	Better survival among non-obese
p21 loss	Better survival for patients >60 yrs
COX-2-positive tumours	Increased cancer-specific mortality
18q	Loss in non-MSI → decreased survival No loss → 5-year survival 96%
PI3KCA mutations	Increased survival among chronic aspirin users
Line-1 Hypomethylation	The young age of onset and increased cancer and overall mortality
HIF1	High colorectal cancer-specific mortality
Cathepsin B expression	High colorectal cancer and overall mortality
MSI	Better prognosis and survival than CIN/MSS
Cyclin D1 overexpression	Low colon cancer and overall mortality
BRAF V600E	High cancer-specific mortality
CIMP-High	Low colon cancer-specific mortality
MiR-203	Poor survival among stage IV Caucasians patients and stages I and II CRC patients

MiR-21	stage IV CRC poor prognosis
sTNFR-2 expression	Increased risk of CRC development, the lower risk among those taking aspirin
Interleukin-6	Increased CRC development risk, advanced CRC stage, and a worse prognosis
C-reactive protein	Notably, in lean individuals; it is associated with increased risk of developing colorectal cancer.

2.5. MicroRNAs

MicroRNAs are non-coding small RNAs that control the expression of genes in most living organisms. They are 20 – 26 nucleotides long which are targeting messenger RNA and inhibiting their translation into proteins. Briefly, a miRNA binds to an mRNA depends on the complementarities of about a 7-8 nucleotide, hence called seed sequence, to the 3' untranslated region's target sites of messenger RNAs. As a result, miRNAs have a role as guiding strands for the miRISC “miRNA Induced Silencing Complex”, allowing it to locate and degrade targeted mRNAs before they get translated into proteins (Pasquinelli, A. E., 2012). Micro RNAs are part of the cells post-transcriptional gene regulatory network.

2.5.1. Biogenesis and function of MicroRNAs

During the canonical microRNA biogenesis; firstly the transcription of the micro RNA gene into an initial microRNA transcript (pri-miRNA) by RNA polymerase II action (Lee et al., 2004). The pri-micro RNA constitutes of one or more sequential units that can assemble hairpin structures, the stem of this structure is made of complementary-nucleotides that comprise the ~22 nucleotide mature and star miRNA sequences. The hairpin structures serve as substrates for the RNase III enzyme Drosha (Lee et al., 2003), which, besides the help of its binding partner, "DGCR8" (Kwon et al., 2016), cleaves off the stem-loop with a two nucleotide offset, leaving the precursors miRNA (pre-miRNA) stem-loop. After that, pre-miRNA is carried out of the nucleus by Exportin-5 effect (Bohnsack et al., 2004).

Within the cytosol, the Dicer enzyme splits the pre-miRNA by eliminating the loop sequence, leaving a double-stranded RNA molecule called the duplex (miRNA-

miRNA*), with a 2-nucleotide offset at the 3p-ends (Hutvagner et al., 2001). The miRNA*, or passenger strand in the canonical miRNA biogenesis pathways, is degraded, leaving a ~22 nucleotide long mature miRNA which strives biological function. Deep sequencing of miRNAs displays that the majority of miRNA genes follow this mature-star pattern expressing the 5p or the 3p-strand. However, some miRNA genes express similar read counts for both strands (co-mature pattern). The mechanism by which how one strand is identified over the other is not determined yet (Ameres & Zamore, 2013).

Figure (2-10) illustrating different process stages. Pri-miRNA includes hairpin stem-loop and 5p- and 3p- primary transcript arms. After cleavage of drosha within the nucleus, pre-miRNA includes the hairpin stem-loop. Then transportation of Pre-miRNA into the cytosol; here, the dicer cleaves off the loop sequence, leaving the hairpin stem. The incorporation of the mature miRNA follows them into miRISC complex, and the degradation of miRNA* (Flarmark et al., 2016).

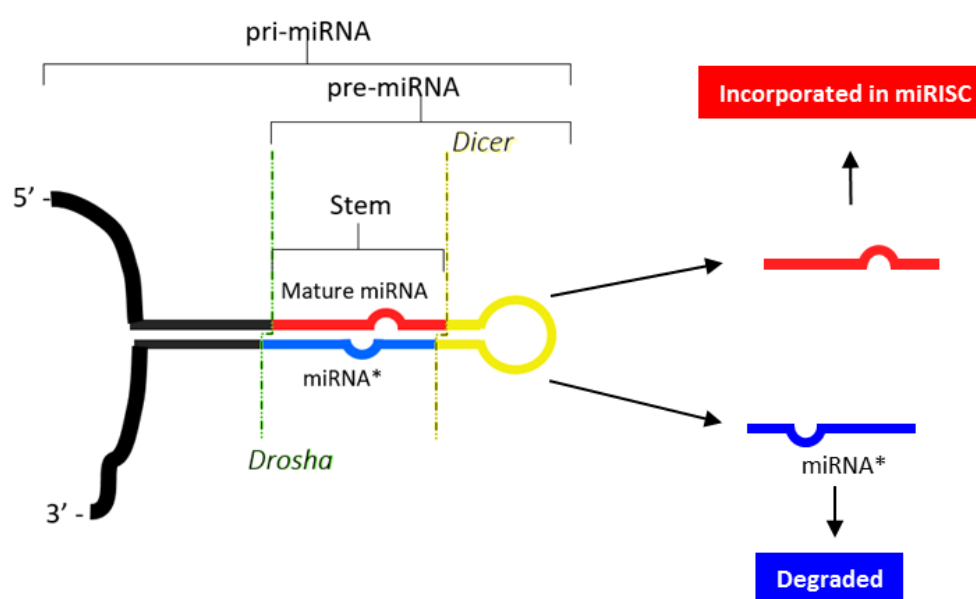


Figure 2-10: Structure of miRNA Canonical miRNA biogenesis pathway

Mature miRNA is later integrated within miRISC and guides miRISC complex to mRNA molecules that have complementary sequences on their 3p-UTR. Argonaute is the crucial protein of miRISC, along with several other protein factors. The expression of miRISC complex silence gene by destabilising or corrupting mRNA or by repressing ribosomal translation (Ramalho et al., 2016).

Both pathways (Conical and non-conical) are presented in (Figure 2-11). It is illustrating that the canonical pathway has a primary transcript, pri-miRNA, split by Drosha, leaving pre-miRNA stem-loop structure. Pre-miRNA is shipped by Exportin 5 into the cytosol, at which "Dicer" cuts off the loop sequence, leaving miRNA-miRNA* duplex. microRNA* is vulgarized, while the microRNA is incorporated within the miRISC complex. The non-canonical pathway starts with a miRNA gene that located inside the coding gene intron. Thus, spliceosome cleavage results in the finished pre-miRNA, no Drosha cleavage required. The remaining steps are identical to the canonical pathway (Krol et al., 2010).

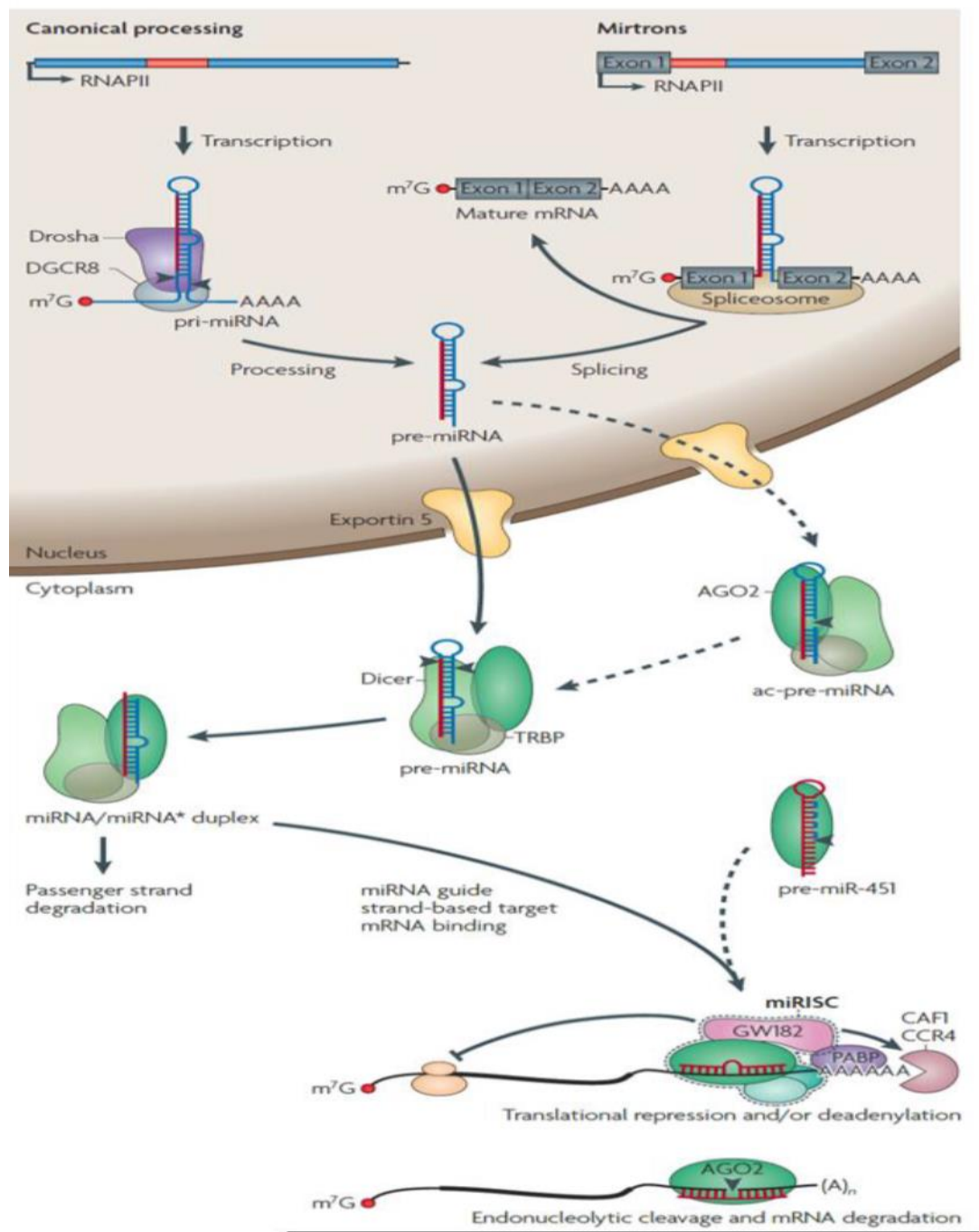


Figure 2-11: The miRNA Biogenesis: canonical and a non-canonical biogenesis pathway

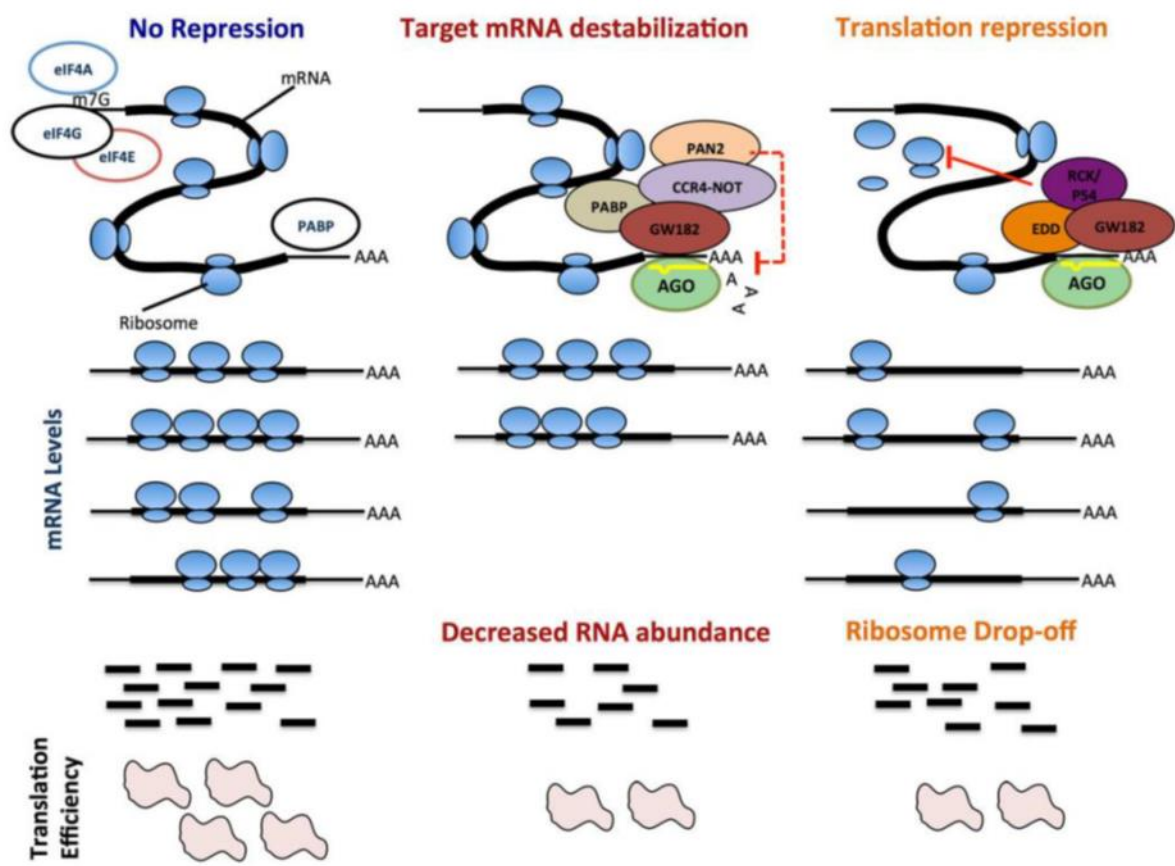


Figure 2-12: MicroRNA Induced Silencing

MiRNA induced silencing: as we can see with no silencing of miRNA, mRNA is abundant and ribosomes free to bind and translate. With miRNA silencing, mRNAs are destabilised, and their abundance drops, while ribosomes are repressed from translation (Figure 2-12) (Ramalho-Carvalho et al., 2016). An animal miRNA to typically induce target of mRNA requires ideal Watson Crick pairing in the 5p-end of nucleotide 2 to nucleotide 7, that is known as “seed” area. Three canonical types of miRNA target sites are present. First, the 7mer-A1; where the Watson crick pairing is formed among the "microRNA seed" with the corresponding messenger RNA purpose site, the objective site has an adenine at position 1. The second is the 7mer-m8; is the site at which Watson crick pairing is formed amidst the messenger RNA target site" and the miRNA 2-7 seed at an eight nucleotide plus. The 8mer site contains Watson crick pairing for the seed and nucleotide 8, and adenine at mRNA position 1. Figure 2-13, representing miRNA targetting, (a) 7mer-A1, with adenine in position 1, and Watson Crick is pairing for

about a 2 to 4 fold alteration in the corresponding protein level (Baek et al., 2008). Although it has a significant role, it is not sufficient to switch the gene off entirely. Instead, they are proposed to operate as an inhibitor of "gene expression", permeating cells into fine-tune their gene expression in different tissues to befits their specific requirement (Bartal, D. P., 2009). Bartel & Chen, (2004) made the analogy to rheostats (Figure 2-14), where miRNA derive a resistance to a gene in either by the amount of miRNAs displayed in the cell or the number of the mRNA complementary and occupancy sites.

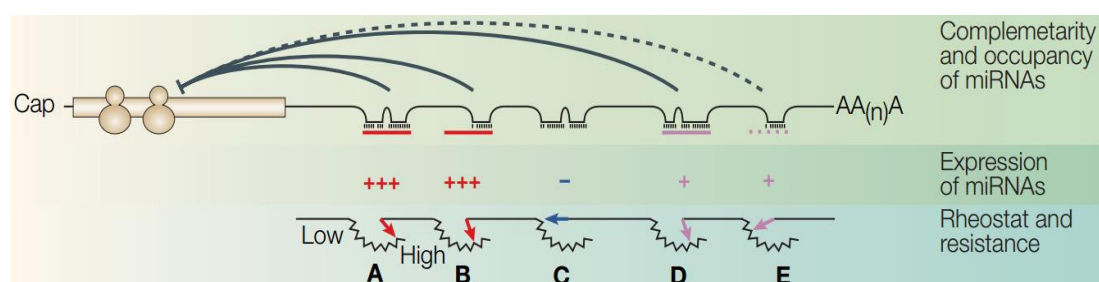


Figure 2-14; Rheostat model of miRNA ‘resistance’ Expression of miRNA and the number of target sites both determine the amount of ‘resistance’ imparted by miRNA.

A has both high miRNA expression and high complementarity and occupancy, imparting high resistance. B and D both also impart high resistance, even though B has fewer occupancy sites, and D has less miRNA expression. E has low imparted resistance due to low miRNA expression and few occupancy sites, while C has no imparted resistance even with abundant complimentary sites since no miRNAs are expressed.

Since a single mRNA UTR may target sites at multiple miRNAs; and in contrary, a miRNA may target several mRNAs; it may be supposed that there are regulatory networks mediated by elaborated miRNA, which permit precise fine-tuning of expression levels not possible by the standard, more crude, transcription factors. Moreover, some RNA molecules have ample target sites of miRNA and therefore can bind a lot of the miRNA are available in the cell, leaving very few available to suppress the rest RNA with those target sites. Such interconnections of miRNAs with ceRNAs (competing endogenous RNAs) have been suggested as a vital new mechanism (Salmena et al., 2011).

Concisely, the only limited number of miRNAs is available to repress mRNAs at a single time. The number of RNAs containing attaching regions for a given microRNA in the cell will influence their ability to suppress a specific mRNA. In this manner, all

RNA molecules that have same microRNA's target sites would challenge with each other for repression, allowing for a mechanism whereby different separated genes could interact with each other, producing an elaborate competitive endogenous RNA regulatory networks. CeRNAs were proposed to be functionally critical in muscle cells, where long-noncoding RNA linc-MD1 serves as ceRNA for miRNA that is regulating differentiation of muscle (Cezana et al., 2011). As well as regulate the differentiation in glioblastoma (Sumazin et al., 2011), prostate cancer in men (Tay et al., 2011) and malignant melanocytes carcinoma (Karreth et al., 2011). In the previous studies, they found that ceRNA influence the miRNA complement available to regulate PTEN, a tumour suppressor gene. On the other hand, recent studies doubt the physiological relevance of such a ceRNA, assuming that the probability of noticing such a ceRNA effect is much lower than initially expected (Denzler et al., 2014; 2016).

2.5.3. Evolution of miRNAs

During the eukaryotic evolution, RNA interference (RNAi) is early originated. They analysed the RNAi machinery key members in five eukaryotic 'supergroups' and reported that all had at least one of Piwi-, Argonaute-, or Dicer-like proteins, and single RNA-dependent RNA polymerase. Hence, they suggest that RNA interferences were previously existing in the latest common progenitor of eukaryotes, likely originating as a defence mechanism against transposable elements (Cerutti & Cases-Mollano, 2006). This machinery would have the capability of degradation of the transcript. However, recent evidence proposes several separate developments of miRNAs and their processing machinery in animals and plants.

MiRNAs would be a part of the foundations upon which more abundant, more complex organisms could emerge. A miRNA plays a beneficial evolutionary regulatory effect on a gene transcript would be preserved through evolution. If said miRNA, or the target site(s) on the corresponding gene transcript, were to experience a mutation that eliminated the beneficial regulatory effect, it might have a negative effect.

Interestingly, other genes might undergo a mutation in their 3'-UTR, permitting the same miRNA to exert a regulatory effect upon them as well. If the regulatory effect

were beneficial, the mutation would be conserved via evolution. Gradually, a complex miRNA gene regulatory networks would develop as more miRNA genes arise, and more genes would be under their control (Tarver et al., 2012).

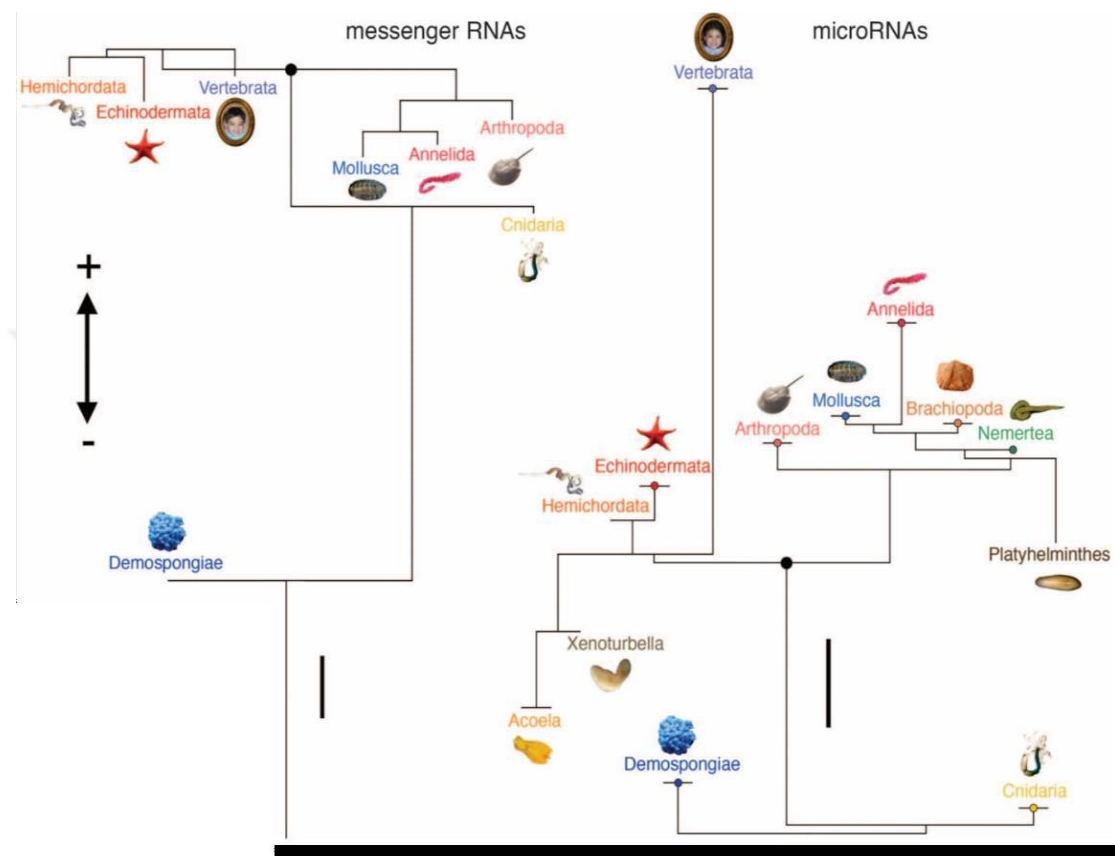


Figure 2-15: MicroRNA and Tissue Complexity

Figure (2-15) is illustrating the Plot of mRNA genes acquisition (left) and miRNA genes (right) throughout the advanced complex animal lineages. Branches indicate gene acquisition; lineages going up have more genes, lineages going downward have fewer. Scale bars correspond to 10 genes. The miRNA complements rapidly increase from demosponges to cnidarians and bilaterians, after which the complement remains more or less flat. Mirna complement, meanwhile, sees great gains in the bilaterian lineage compared to the cnidarian. Increasingly complex species see increasing miRNA complement. Xenoturbella and Acoela groups have undergone simplifications in their

morphology and complexity, and see a drop in their miRNA complement (Erwin et al., 2011).

2.5.4. Annotation and nomenclature

The arrival of next-generation sequencing has resulted in a considerable increase in the announced miRNA. MiRBase is the current repository for remarked miRNA (Kozomara & Griffiths-Jones, 2013), encloses about 1,917 entries of human miRNA. MiRBase is not a curated database, and it is suspected that a large proportion of entries are false annotations.

To confirm the validity of the human miRNA complement in miRBase by establishing a batch of criteria to define miRNAs, then compare the human entries in miRBase against those criteria. By applying the criteria to all miRBase entries, it is illustrated that less than two-thirds of all human entries fulfilled the criteria. Thus, a new curated open access miRNA gene database, 'MirGeneDB' was established to provide a high-quality repository, correctly annotated miRNAs. Ambros et al. (2003) introduce The conventional miRNA naming by providing each miRNA with the prefix "miR", accompanied by a number based upon the sequential order in which the miRNA was discovered. miRNAs with highly related sequences have the corresponding names, given a number or letter suffix, to differentiate between them.

Similarly, their coding genes are named, other than conventionally using capital and italics letters. However, this system of naming does not take into account what evolutionary relationship between miRNAs. Accordingly, a revised nomenclature system was achieved in where orthologous and paralogous miRNAs can be identified based on their name.

In order to discriminate between the new and the old nomenclature system, gene names start with uppercase miR-, and miRNA families use total capital (MIR-). Annotation of paralogous genes within a family is with a P followed by a number, initiating with the 1st member of the family. If a second duplication event has occurred, the P letter and number designation are continued by a letter indicating the sequence of

duplication. Lastly, all orthologues in all species are given the same name, to avoid confusion when comparing miRNA between species.

2.5.5. Selected Micro-RNAs

2.5.5.1. Mir-195

According to the gene base in NCBI, micro-RNA-195 is located on chromosome 17p13.1. In human cancer, this area at which miR-195 is located is usually deleted (Su et al., 2016). MiR-195 is induced by stress and activated in several diseases including heart failure, cancer and schizophrenia. Micro-195 belongs to the miR-15/107 cluster (Finnerty et al., 2010). MiR-195 is one of the widely studied microRNA molecules, as its alternated expression has roles in multiple tumours onset and progression. Many studies have proposed low expression level of miRNA-195 in several tumours for instance in thyroid cancer (Wang, F et al., 2015), gastrointestinal tract cancer (Deng et al., 2013; Yang & Song, 2015; Wang, M et al., 2015), glioblastoma (Susluer et al., 2015), cancer of the prostate (CaiC. et al., 2015), cervical cancer (Li et al., 2016), bladder carcinoma (Iteseako et al., 2014), and osteosarcoma (Han et al., 2015). Moreover, serum miR-195 was proposed as a likely bio-indicator for malignancy discovery and outcome prediction in breast malignancy (Zhao et al., 2014), adenocorticoid cancer (Feigo & Cherradi, 2013) and osteosarcoma (Cai, H. et al., 2015). Decrease level of tissue miRNA-195 expression was detected in a patient with NSCLC, and there was a correlation between its downregulation with shorter overall survival (Liu, B. et al., 2015). It is also has been associated with lung cancer (Yu et al., 2018), colorectal cancer (Feng et al., 2018), and pancreas cancer. Furthermore, miR-195 downregulation was correlated with the prostate cancer's onset, progression, prognosis and drug resistance (CaiC. et al., 2015; Ma et al., 2018).

They were represented via bioinformatics analysis the complementary attaching regions connecting microRNA-195 and 3'-UTR of MAP2K1 messenger RNA, proposing potentially targeted regulation between them. Cai, C. et al., (2015) administered that miR-195 up-regulation can suppress prostate cancer cells migration or invasion potency through targeted inhibition of expression of RPS6KB1 to facilitate cell apoptosis, and

suppress the growth of tumour cells in BALB/c nude mice to suppress cell invasion or infiltration. Similarly, Guo et al., (2008) addressed that miR-195 over-expression marks and represses the creation of BCOX1 gene, suppress proliferation, transfer and aggression of cultured Prostate Cancer-3 / LNCaP cells, thus decrease in-vivo tumorigenesis and metastasis. Wu et al., (2018) found that weaken migration or invasion potency of prostate cancer cells via targeting regulating of Fra-1 as a result of the overexpression of miR-195.

Various studies have illustrated that the microRNA level of expression is strictly correlated to tumour breakthrough and upheaval. Former researches explained the abnormal pattern of expression of several microRNAs in Colon Cancer tissues, and "miR-195" is an example of them (Du et al., 2015; Zhou et al., 2016). Part of the investigations was conducted to clarify the function of miR-195 in Colon Cancer, which considered that microRNA-195 roles as a tumour inhibiting microRNA in colon cancer (Song et al., 2017).

The miR-195-5p might direct the MMP14, and affect the Tumour Necrosis Factor signalling pathway in Colorectal Cancer. Similarly, regarding Colorectal Cancer, Liu, B. et al. (2015) observed that microRNA-195 is considerably low in Colorectal cancer excised specimens and cell lines in comparison with neighbouring non-cancer tissues and healthy cells. Furthermore, it has been confirmed that the up-regulation of miR-195 can decrease the colorectal cancer cell proliferation through CDK2 and CDK8 down-regulation (Luo et al., 2016).

Additionally, promoting programmed cell death as well as reducing the viability of CRC cells via suppressing the expression of BCL-2 anti-apoptotic protein (Yang et al., 2015). Wang et al. (2012) stated the significant microRNA-195 decrease in patients with metastasises and an advanced tumour stage. It has been found that miR-195 down-regulate CARMA3, commencing to the restraint of CRC cell reproduction (Wang et al., 2012). CARMA3 is assumed to perform an essential task in cancer development (Zhao et al., 2013).

The Signaling pathways, for instance, the Hippo/YAP pathway, operates significant parts in the progression of CRC (Su et al., 2017), akin to the performance of

miR-195 reported in Hepatocellular Cancer (Yu et al., 2017). Earlier researches showed that "FGF2" has a crucial function in extension, differentiation and capacity of Colorectal Cancer cells, and as previously mentioned, Wnt signalling pathway is unit of as the crucial pathway in growth development and metastasis (Jibiti et al., 2014; Li, Z. et al., 2016).

It has exposed that the anti-proliferative role of miR-195 is exerted by affecting and obstructing the Wnt/ β -catenin pathway (Zhang X et al., 2016). Recently, the miR-195 expression has a negative relation with IGF2BP2 and RAF1, and RAF-1 messenger RNA cohesion regulates by IGF2BP2 via modifying miR-195 assembly in Colorectal carcinoma (Ye et al., 2016). RAF1 is one of the MAPK pathways and can be connected by miR-195 at its 3'-UTR (Chong et al., 2003).

Recent research explicated that miR-195 refines Colorectal Cancer cells to radiation by gene regulation implicated in cell proliferation and survival, for instance, on CARM1 gene (Zheng et al., 2017). CARM1 (PRMT4) is able to exert oncogenic and tumour-suppressive employment (Wang et al., 2014). Other recent investigations have also reported a notable association amongst microRNA-195 and the guard of drug resistance. miR-195 was manifested to sensitise the Colorectal Cancer cell lines to doxorubicin via directly targeting BCL2L2, that is a critical antiapoptotic control by overcoming apoptosis (Qu et al., 2015).

The origin of the miR-195 gene is from intron-7 that found on chromosome 17p13.1 and the reverse strand of the messenger RNA gene AK098506, encoding an anonymous working protein (MiRBase). The prophesied stem-loop structure of miR-195 is defined using the miRBase is presented in (Fig 2-16). The hairpin of miR-195 gives origin to the miR-195-5p & miR-195-3p, which are guide strand and sister strand/passenger, respectively (Flavin et al., 2009; He et al., 2011). The following figure (Fig 2-17) illustrates the miR-195 roles in different pathways in malignancies and the indicated target genes. Mir-195, as we can see, inhibits the proliferation and invasion via inhibiting WEE1 signal. On the other hand, it induces the apoptosis via, for instance, the inhibition of BCL-2 and WNT3A. Also, the role of targeting retinoblastoma and E2F3 genes is indicated; (Yu et al., 2018).

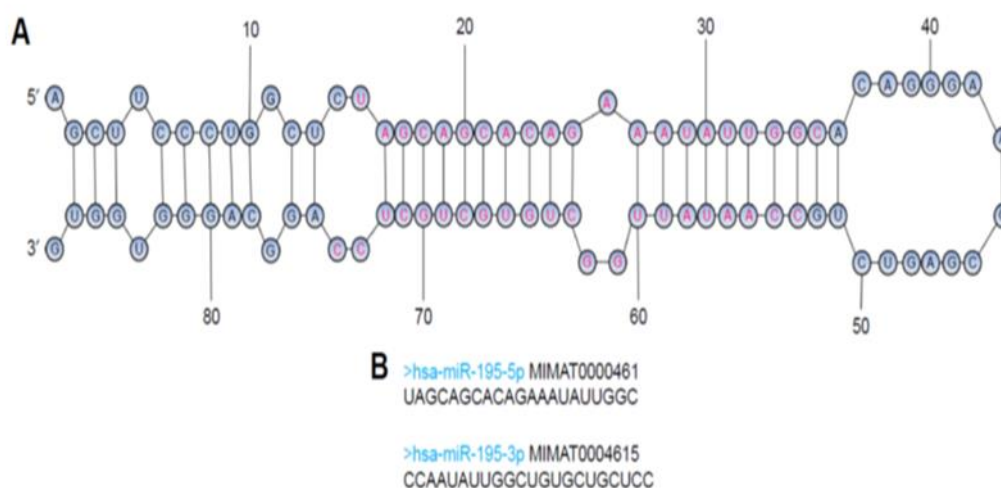


Figure 2-16: The predicted miR-195 stem-loop structure is determined using the “miRBase.”

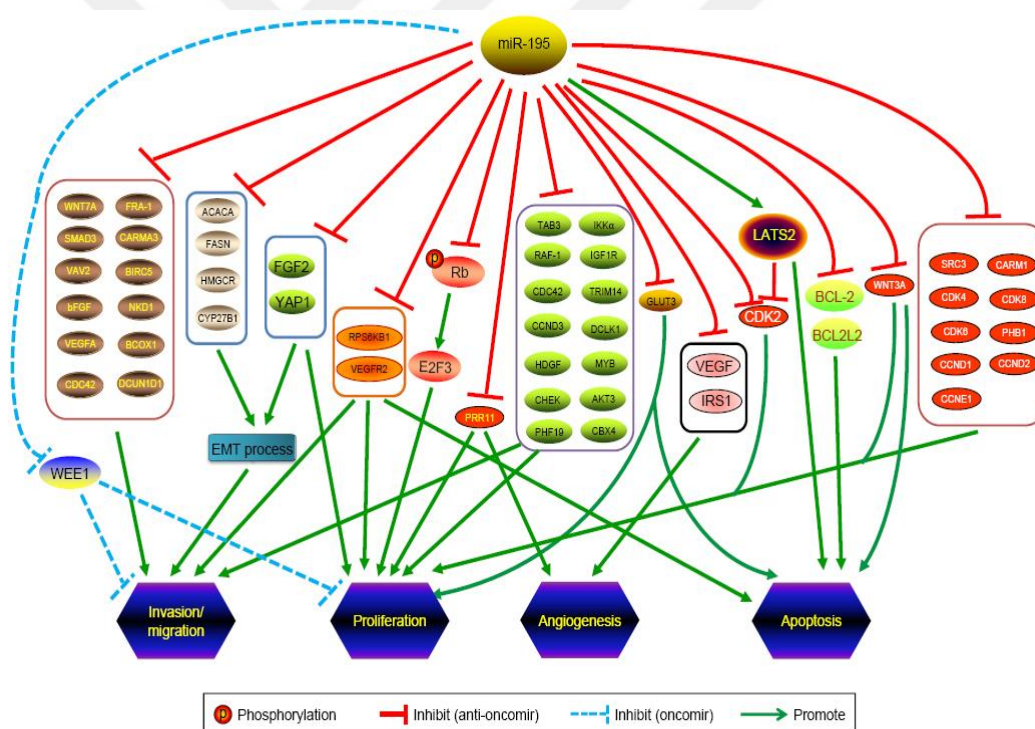


Figure 2-17: The roles of miR-195 in the progression and development of malignant tumours and its target genes

2.5.5.2. Mir-126

It is an endothelial cell-specific miRNA. Chromosome 9 is the site where pre-miRNA-126 is located, from which MiRNA-126-3p and -5p are originated, and both were recognised as critical physiological agents in the development of certain cancers (Zhang et al., 2013). The gene is found in an intron of a protein-coding gene, EGFL7, and its expression was earlier administered to be intimately associated with the T2 EGFL7 transcript (Zhang et al., 2013). Downregulated miR-126 level was found in cancer tissue; the circulating MiR-126 was suggested to be utilised as a simple biological indicator for the differentiation of benign from the malignant type of nasal neoplastic pathologies (Tomasetti et al., 2018).

Moreover, control of miR-126 and its EGFL7 target gene associated with Methylation was addressed in several neoplasms, such as oesophageal squamous carcinoma, glioma and malignant pleural mesothelioma (Cui et al., 2016). The results showed miRNA-126-3p and -5p reduced display in Lung adenocarcinoma and even lower in the later stage of cancer and metastasis, miRNA-126 has an important task in LUAD growth (Chen et al., 2019).

The structural organisation of egfl7 gene and their products (Fig 2-18); It constitutes of 10 exons, just the third and ninth exons cipher the EGFL7 protein. The protein exhibits a modular architecture which has SS (N-terminal signal secretion peptide) pursue by an EMI (Emilin-like domain), then 2 EGF, and a coiled-coil region. The pre-microRNA is positioned in intron 7 of the egfl7 gene from its miR-126, and -126* arise. Those microRNAs are incredibly preserved in throughout the evolving within various kinds (Meister & Schmidt, 2010). Structural organisation of egfl7 gene and their products.

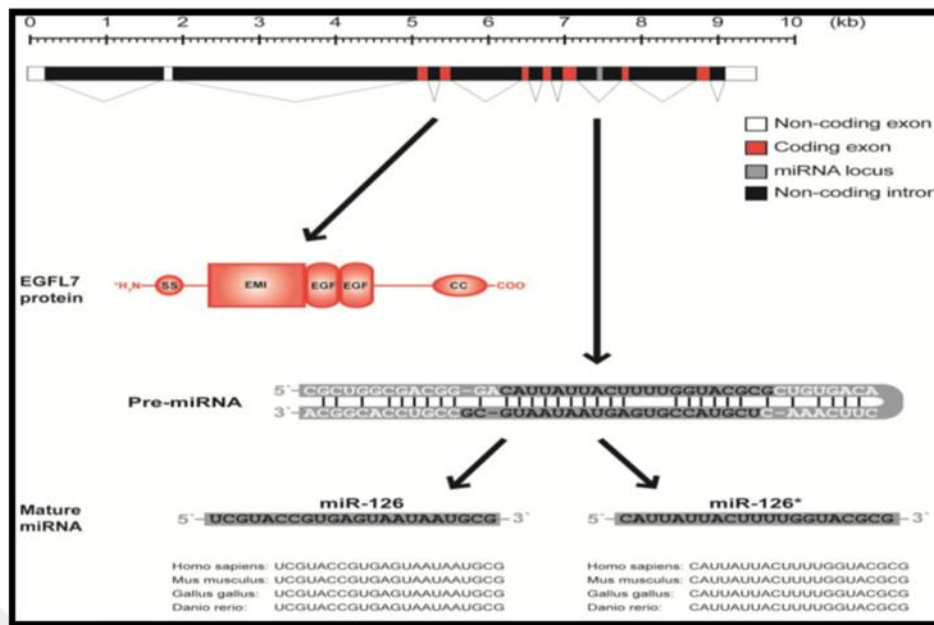


Figure 2-18: Structural organisation of *egfl7* gene and their products

The state of the miR-126 display was remarkably more depressed in subjects with stage III-IV malignant glioma than in cases with stage I-II, using miR-126 inhibitor significantly increased the invasiveness of the malignant cell, compared with control, in this study they concluded that miR-126 by inhibiting MTCP1 reduces the immigration of malignant glioma cells (Han et al., 2018). As reviewed in Han et al., (2018) study miR-126 regulates its associated genes, for instance, CRK Proto-Oncogene, Adaptor Protein (Crk), VCAM-1, and CRK Like Proto-Oncogene Adaptor Protein (CRKL).

Additionally, the enhancement of miR-126 expression disabled cell-cycle entrance, which leads to markedly diminished hematopoietic participation. In response to extraneous signals, miR-126 attenuates signal transduction, by regulating various targets within the PI3K/AKT/GSK3b pathway; miR-126 performs a crucial function in constraining cell-cycle progression of Hepatocellular Carcinoma stressing on the significance of miRNA in HSC function regulation (Lechman et al., 2012). The action of microRNA-126/microRNA-126* in formation of new vascularity in tumour appears reasonable. Alteration in the expression level of these microRNAs was published in different carcinoma cells arising from the breast (Negrini & Calin, 2008), lung (Crawford et al., 2008), cervix (Wang et al., 2008), liver (Ladeiro et al., 2008), prostate (Musiychenko

et al., 2008), colon (Hu et al., 2008), oesophageal (Hu et al., 2011), or stomach carcinoma (Li et al., 2010), also in leukaemia (Li et al., 2008).

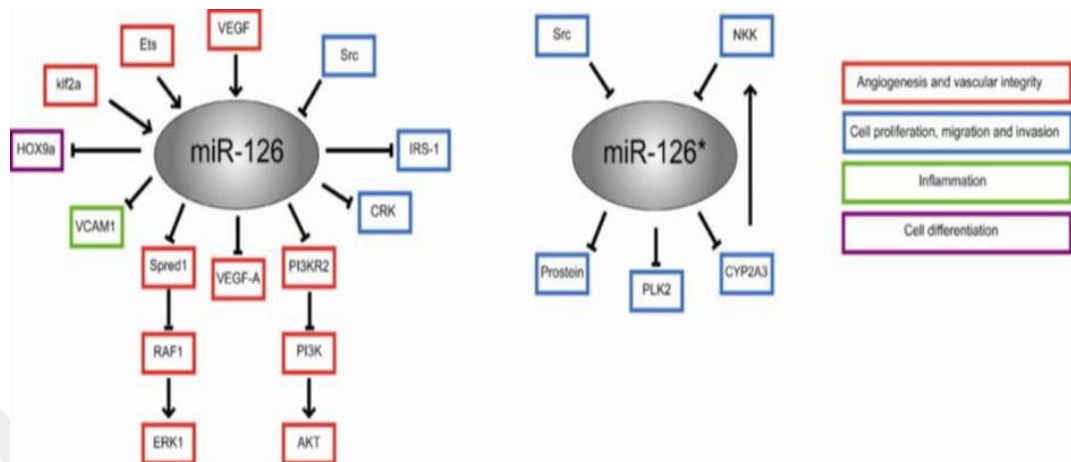


Figure 2-19: Schematic of miR-126/miR-126* signal pathways and affected cell functions

The schematic of miR-126/miR-126* signal pathways and affected cell functions (Fig 2-19); Various factors guide the expression of miR-126/miR-126* throughout the angiogenesis means and the advancement of the tumour. miR-126 expression creates an encirclement of signalling pathways which direct new vessel formation, the cohesion of vessels, inflammatory process, and discrimination; moreover, miR-126 and miR-126* may serve as tumour inhibitor through the repression of propagation, movement, an incursion of the tumour (Meister & Schmidt, 2010).

MiR-126 down-regulation in malignant vs healthy tissues was proclaimed for malignancies of the lung (Cho et al., 2009). Within colon carcinoma, they submitted miR-126 as a tumour suppressor and proposed a relationship linking miR-126 decline and disabled signalling through PI3K by its regulatory subunit p85 β down-regulation (Guo et al., 2008).

The following figure (Fig 2-10); (A) part illustrates that miR-126/miR-126* at high levels direct conventional vessel configuration via intonation of the endothelium response to vascular growth factors and limitation of leukocyte adhesion. While (B) Lack of the tumour-inhibitor microRNA-126/microRNA-126* throughout carcinoma

aggression may end in raised neoplasm cell survival, proliferation, and migration; magnified the development of metastasis, and increasing leukocyte adhesion and leukocyte invasion into the vessels wall. Moreover, lack of microRNA-126/microRNA-126* might disrupt the integrity of the vessels, generating a disorganised and unnatural tumour vascularisation (Meister & Schmidt, 2010).

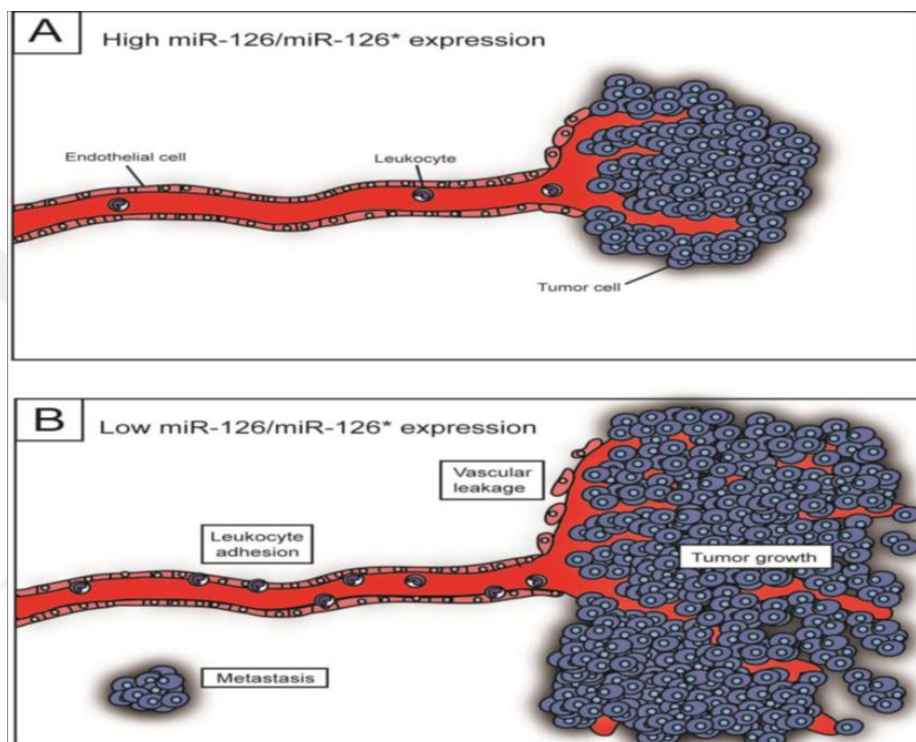


Figure 2-20: The Putative effects of miR-126/miR-126* on the cancer progression

2.5.5.3. Mir-92 a

MicroRNA-92a-1-5p, is one of microRNA-17-92 family, it is significantly up-regulated in stomach cells up on bile acids prescription and produced a phenotype similar to intestine criteria. Concerned to those issues and the current results proposing the function of miR-17-92 members in gastric Intestinal Metaplasia and tumour, this cluster was formally exposed to be over-expressed in numerous classes of neoplasms (Li et al., 2017). MiR-17-92 group was found to be overexpressed in the serum of gastric carcinoma and intestinal metaplasia subjects (Mihailovich et al., 2015). The mature miRNA-92a sequence is (UAUUGCACUUGUCCCGGCCUGU), with a miRBase

accession number MI0000093 (Mihailovich et al., 2015; Zagorac et al., 2016). Some studies revealed that via affecting KLF2 and KLF4, the miR-92a provoked inflammation of endothelial cells (Liu et al., 2016), or by the NRF2-KEAP1-ARE signalling (Liu et al., 2017), and controlling levels of cholesterol and Golgi volume through protein secretion (Loh et al., 2017).

The elicitor for discrimination of the intestine at the molecular level in the gastric circumstances is the display of transcription factor specific to the intestine that maintains and leads differentiation of growing intestines, also in both Barrett's metaplasia and gastric IM (intestinal metaplasia). In IM, CDX2 mediates transdifferentiation via increasing transcription of intestinal markers for instance; VILLIN (VIL1), Krüppel-like factor4 (KLF4), sucrase-isomaltase (S-I), and MUCIN 2 (MUC2). In regards to Barrett's metaplasia, the display of CDX2 might also be raised with additional definite transcription factors, for instance, KLF4 and CDX1, often by stimulation of (NF κ -B) pathway responding to prolonged bile backflow. Nevertheless, how could the backflow breaks the balance of stomach epithelial cells and the pathogenesis, causing the elevation of "CDX2" in gastric Intestinal Metaplasia still mostly undiscovered (Li et al., 2019). It explicated that the level of circulating microRNA-92a level was markedly enhanced even in a mouse is carrying human CC xenografts. Such data confirm the potential application of miR-92a/MVs as a new biological determinant for the disclosure of colonic malignancy as early as possible (Yamada et al., 2013).

MiR-92a constrains the propagation of ovarian carcinoma cells to peritoneum by repressing integrin $\alpha 5$ display, miR-92a is found at decreased levels in ovarian carcinoma, that display raised levels of integrin $\alpha 5$. Nevertheless, it is produced at higher level in RMUG-S cells, which has a depressed state of integrin $\alpha 5$. MiR-92a-1-5p was influenced by bile acids, that is FXR antagonist GS prohibited inducement, proposing the receptor of bile acids (FXR) directed increase level of miR-92a-1-5p in stomach cells. The alteration in miR-17-92 family was earlier documented to be correlated with intestinal metaplasia and tumorigenesis of the gastric tissue. Recently conducted researches further intimated that continued raised levels of onco-microRNAs, counting the members of miR-17-92 cluster might have a partial contribution to the collapse of intestinal metaplasia inverting treatment after *Helicobacter pylori* elimination. These

findings provided pieces of evidence that microRNAs 17–92 cluster components may significantly act in increasing the intestinal metaplasia evolution in gastric tissue of an individual complaining from bile reflux (Ushio et al., 2019).

Li et al., (2019) found an elevation of multiple members of microRNA 17–92 family among gastric cells exposed to bile acids in a microRNA microarray; it is the initial research that shows evidence that bile acids as a carcinogenic agent for gastric malignancy together with *Helicobacter pylori*, that increases the expression of CDX2.

2.5.5.4. Mir-214

Dynamin-3 gene's intron number 14 contains miR- 214 on chromosome 1q24.3, miR-214 is identified to regulate several tumour-linked signalling cascades in various malignancies. MiR-214 reportedly operates a function in different tumour classes and is involved in multiple pathways (Penna et al., 2015). As reviewed through Cagle et al.,(2019), the function of miR-214 is specific to cell type, its various capacities relying on its distinct target genes. miR-214 might be either tumour suppressor or oncogenic functions in different cancer types, including ovarian malignancy, melanoma and breast cancer. miR-214 in malignancy of the cervix acts as a tumour suppressor, inhibiting the proliferation, transfer, intrusion of the cells and enhance response to the drug via decrease of MEK3, JNK1, FOXM1, Bcl2, and HMGA1; similarly, miR-214 through regulating Caspase 1-mediated pyroptosis inhibits cell proliferation and migration of gliomas and brain. Overexpression of miR-214 in breast cancer provokes both oncogenic activities through affecting the PTEN-PI3K/AKT signalling and tumour suppressor effect via affecting the RFW2-p53 cascade, repressing cell proliferation and migration, and favouring apoptosis.

Moreover, some research demonstrates that miR-214 might serve as a tumour suppressor in prostate carcinoma via modulating protein tyrosine kinase 6 (PTK6) and might be utilised as a diagnostic marker and remedial destination. Several pieces of research have revealed that it operates a growth suppression function in colon malignancy (Long et al., 2015) and could connect to ARL2's 3'UTR. Besides, Necl-2 can be mediated by miR-214, MicroRNA 214 control ErbB2/ErbB3 signalling pathway. It has been

manifested that the expression of miR-214 drops in 5-FU-immune colon malignancy and miR-214 knockdown profoundly provokes cell resistance to 5-FU via acting on Heat Shock Protein-27. They are suggesting that miR-214 holds promising expectation as a therapeutic assistant for the therapy of carcinoma of the colon.

Additionally, SNHG6 overexpression has been connected to Colorectal Cancer progression and prophesied bad outcomes. It is reported that "SNHG6" used its oncogenic capacities via cadging miR-26a/b and miR-214 and playing as a ceRNA (challenging internal RNA) for EZH2 (Xu et al., 2019). It is pointed that miR-214 hindered the growth-inducing impact of CAFs on gastric cancer via affecting FGF9 within CAFs, and the EMT manner regulation in Gastric Cancer cells, claiming miR-214/FGF9 in CAFs as a promising target for treatment procedures in GC (Wang R et al., 2019).

Zhao et al., (2018) claimed that miR-214 downregulates Dact2 gene expression, stimulates Wnt/ β -catenin signalling pathway, also encourages gastric cancer proliferation, migration and invasion. miR-214 and TFAP2A constitute the most abundant divisions to co-control several genes, and they have established cooperation alongside Hematopoietic cancers and cancer-like stem cells growth. miR-214 roles in repressing TFAP2A, NRAS and FSCN1 (Mohamed et al., 2019). MiR-214-3p has the capacity to act as a biological indicator that can discriminate Hepatocellular Carcinoma from non-Hepatocellular Carcinoma subjects (Jin et al., 2019).

It has been shown that HOXA11-AS and miR-214-3p might sceptically control ones another. MiR-214-3p was presented to be reduced in oral SCC. Moreover, they additionally reported that PIM1; protooncogene serine/threonine-protein kinase is an aim of miR-214-3p. The expression of PIM1 might be reduced by miR-214-3p and induced through HOXA11-AS. Wang et al. findings emphasised the relevance of HOXA11-AS/miR-214-3p/PIM1 axis in the Oral Squamous Cell Carcinoma medication endurance and proposed likely purposes for promoting chemotherapy of Oral SC carcinoma (Wang, X. et al., 2019). It has been revealed that the upregulation of microRNA 214 in osteosarcoma engages to radio-resistance via adjusting the PHLDA2/Akt pathway as it decreases the PHLDA2 expression through aiming to its 3'-UTR (Li et al., 2019).

PC-3-derived exosomes downregulated miR-214 and the genes of mature osteoclasts, such as CTSK, NFATc1, ACP5 and via blocking the NF- κ B signalling pathway, leading to inhibiting osteoclast differentiation. Thus, increase miR-214 states in the site of metastasis of the bone may inhibit the prostate cancer infiltration (Duan et al., 2019).

LINC01535 upregulation correlated with poor outcomes in cervical carcinoma. LINC01535 encourages cell expansion, transfer and incursion of the cervical tumour through subjugating the microRNA 214/EZH2 managerial loop (Song et al., 2019). Yao et al. showed that DANCER is elevated in malignant prostate tissues and cell lines. DANCER might increase cell outgrowth and metastasis in vitro and in vivo. Also, it serves as a sponge of miR-214-5p" at a molecular level, in turn affecting E2F2 expression. They proposing that, DANCER/miR-214-5p/E2F2 pathway modulates the development of PC, which might be a possibility to be a therapeutic target for PC (Yao et al., 2019).

MiR-214 hinders AEBP1 action and enhances the responsiveness of chemotherapy in CRC; AEBP1 is anticipated to display a value for the indicator of recurrent Colorectal Carcinoma metastasis and is a possible molecular target for therapy; AEBP1 is a variety of inflammation-associated regulatory factor inhibiting the κ B inhibitor, I κ B α , concerning encourage the activity of the nuclear factor (NF)- κ B pathway, and implicated in the occurrence of several tumours (Li & Fang, 2019).

Yang et al. (2019) study proposed that CACR may be an innovative curative object via the CACR/microRNA 214-3p/caspase-1 pathway as they found that, miR-214-3p knockdown partly reversed the advantageous influences of CACR silencing on pyroptosis in cardiac muscle cells. The reduction of miR-214 level, significantly repressed the breast cancer cells survival, immigration and invasiveness, and inhibited the PI3K/Akt/mTOR route (Zhang, Y. et al., 2019).

TargetScan prediction affirmed that miR-214 holds a binding position to TGM2; MiR-214 by targeting transglutaminase-2 gene (TGM2), inhibits the growth, immigration and intrusion of CRC cells. A contradictory relationship within microRNA 214 and TGM2 manifestation were ascertained in Colorectal Cancer. MicroRNA 214 overexpression increases E- cadherin and tissue inhibitor of metalloproteinases-2

expression and decreases MMP-2 and MMP-9 expression, that inhibiting cell invasion and migration; Also, PI3K and Akt phosphorylation is inhibited by microRNA 214 (Shan, Zhou & Chen, 2019).

The interaction network of the proteins, UQCRC1 has been revealed to be a critical gene and a possible target gene of miR-214-3p. Significantly, miR-214-3p is elevated, while UQCRC1 was decreased, in osteosarcoma specimen and cell lines. Down-regulated microRNA 214-3p expression stimulates the expression of UQCRC1 and suppressed OS cell proliferation and vice versa. That means high miR-214-3p expression may promote OS cell proliferation by targeting UQCRC1, providing insight into a potential target for preventing and treating osteosarcoma (Zhao et al., 2019).

2.5.5.5. Mir-224

MiR-224 operated via the cooperation with apoptosis inhibitor-5 transcript expression in order to control cancerogenesis (Wang et al., 2008). Considering then, miR-224 is claimed to be at a lower state in several cancers. MiR-224 elevated in cervical carcinoma, glial cell tumour, non-small cell lung malignancy, and CRC and reduced in prostate tumour, intimating a double regulative function performed via miR-224 both as oncogenic factor or a tumour suppressor gene.

Functional studies at the molecular level on miR-224 have revealed that miR-224 controls tumour propagation by various signal transduction pathways, for instance, cell cycle progression and NF- κ B as well as TGF- β signalling pathways, via straight communications with multiple tumour-related genes such as PHLPP1 and PHLPP2 in CRC, SMAD4 and TNFAIP1 in Non-Small Lung Cell carcinoma, and TPD52 in prostate carcinoma. Additional researches proposed that miR-224 might be a predictor for chemoresistance or radiation sensitivity. These findings documented the crucial task of miR-224 dysregulation in the advancement of malignancies (Zhang, L. et al., 2019).

Wang, G. et al., (2019) found that miR-224 may operate crucial tasks in the molecular roles of nutrient-depleted cells probably via controlling the objective PTEN and downstream signal PI3K, inferring the possibility of using miR-224 as an aim for lung cancer treatment. MicroRNA 22 is further reported to be a pivotal agent for infirm

prognosis and chemo-resistance. The in vitro trials exhibit that miR-224 increases propagation and aggression of cells. On the contrast, other studies recognised a more depressed level of microRNA 224 display in Non-Small Cell Lung Cancer tissues.

It has been confirmed the display of miR-224 in NSCLC specimens; by analysing the consequences of miR-224 on the cell survival, programmed cell death, and apoptosis of A549 cells under serum-lack medium situation; and examining the potential signalling pathway included in those duties of the cell, and ultimately distinguished PTEN as its primary target. Aforementioned will enable us to thoroughly comprehend the performance of miR-224 throughout its pathological roles and approaches in A549 cells under the effect of the serum-starved situation (Wang, G. et al., 2019).

In another study, they demonstrated the close utilitarian diversity of CASP9 within the miR-224 control, that influences cell proliferation, migration, and invasion due to caspase-3/7 activities. The oncogenic effect of microRNA 224 was recognised in mammary Carcinoma, in which miR-224 overexpression leads to suppressing CASP9 and in turn leads to the apoptosis pathway inactivation. In turn, this results in promoted proliferation, immigration, and infiltration of the breast carcinoma cell. This study emphasises the significance of the apoptosis pathway for the offensive behaviour of the condition mentioned above and proposes a possible curative approach for Triple-negative Breast Cancer subjects; also, they discovered that miR-224 is profoundly overexpressed in cells of TNBC malignancy (Zang et al., 2019).

Moreover, It was claimed that miR-224 has an effect in the growth and aggression of Hepatocellular Carcinoma in the aspect of the direction that it attaches to the region 3'UTR of the genes that are the concern of the manner of cell apoptosis, proliferation, and invasion; Shehab-Eldeen et al., revealed the manifestation of miR-122 was reduced, while the circulating level of miRNA 224 was raised in plasma of Hepatocellular Carcinoma subjects in comparison to cases with liver cirrhosis and healthy individuals also both show a correspondence with the neoplasm size. Also, they have better diagnostic accuracy in distinguishing cases with Hepatic cancer from those who do not have HCC. Thus, it might be an encouraging indicator for the determination of Hepatic Carcinoma. Nevertheless, further investigations, including substantial numbers of applicants having various

predisposing causes of cancer, are required before being clinically applied (Shehab-Eldeen et al., 2019).

Zheng and colleagues (2019), by interpretation of Cancer Genome Atlas data, outlined that more depressed expression levels of miR-224 and greater display states of its target API5 in glioblastoma multiforme subjects were associated with decreased durability. Additionally, others further exposed that miR-224 elevation might stimulate responsiveness to radiotherapy of cells of glioblastoma, which considered as assuring choices for the glioblastoma therapy. It proposed that the miR-224-5p could assist as an implied object for glioma treatment. Also, repression of mTOR could hinder the advancement of glioma cells, and mTOR bears cooperation with miR-224- 5p. In summary, circPCMTD1 is proved to imply as an essential oncogenic role in advancing glioma stage.

Lian et al., (2019), proposed an illustrated map regarding the roles of miR-224 in oesophageal cancer (Fig 2-21); the explained pathways and target genes could have a relation with miR-24 roles in the progression and invasion of colorectal malignancy; either it is acting as an oncogene or tumour suppressor gene.

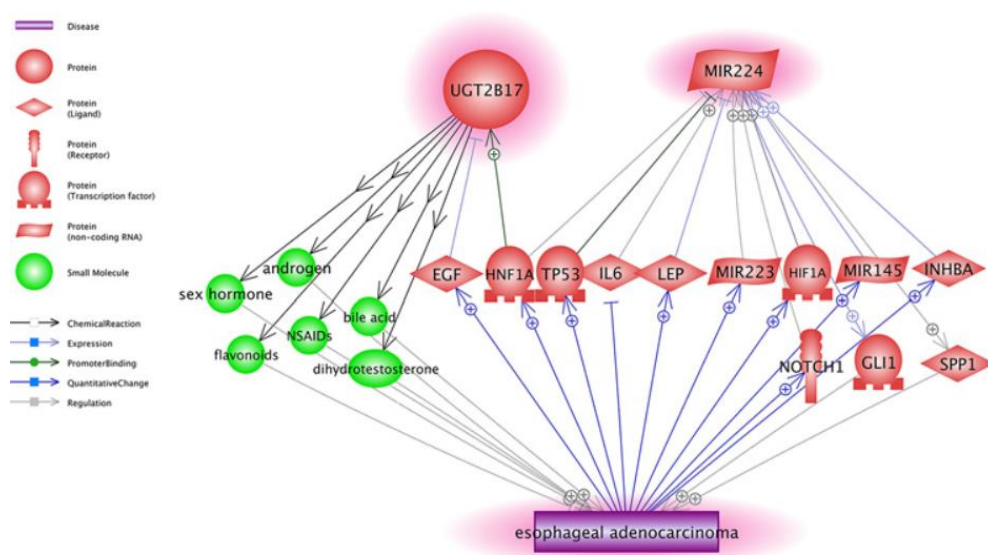


Figure 2-21: The potential pathways that link UGT2B17 and MIR224 to EA

2.5.5.6. Mir-1247

MiR-1247 is one of the miRNAs that have gotten particular awareness in cancer study as its expression is altered in several tumours, such as pancreatic, NSCLC, prostate, hepatocellular, childhood neuroblastoma (Wu, Lin & Xie, 2018), and osteosarcoma (Zhang et al., 2016). It is a tumour suppressor and can also serve as a possible therapeutic factor for various kinds of neoplasms (Shi et al., 2014). The expression level of miR 1247 is significantly changed in pancreatic tissue. For instance, it is remarkably downregulated in the tissues with pancreatic carcinoma as corresponded to similar benign tissues. The increased level of display of miR-1247 was determined to be a predictive factor of prognosis in increasing the overall survival and recurrence-free survival in case of pancreas carcinoma as it is associated with the degree of the tumour at the negative pattern (Shi et al., 2014), i.e. it is downregulated in pancreatic cancer (Zhang et al., 2016).

The expansion, colonies production, tumorigenicity, and cell cycle halt during G0/G1 are inhibited in association with the increase in the level of expression of miR-1247. In pancreatic cancer, it immediately targets neuropilin1 (NRP1) and NRP2, which actively work in neurons, and depresses the growth of the cells. The researchers have also found that all-trans-retinoic acid (ATRA) in low doses may help in redifferentiation and refurbishment of miR-1247 in malignant cells in the pancreas (Shi et al., 2014).

The display of miR-1247 is significantly lowered in the tissues and cells paralleled with NSCLC. On the other side, raised the level of expression of microRNA 1247 with the therapy of 5-azacytidine (5-Aza), inhibits the cell extension, invasion, migration of cells, and cell-cycle progression. In this regard, STMN1, which is entangled in the rule of microtubules and cell cytoskeleton) was claimed to be a functional and immediate objective of miR-1247 because the display of STMN1 is substantially heightened in the cells associated with NSCLC, but treatment with 5-Aza decreases this expression. Furthermore, STMN1 may also increase the invasion and migration of H1299 and A549 cells, which are related to cancerous lung tissues, and upregulation of miR-1247 partially inhibits this activity of STMN1. Researchers have also noted that DNA methylation

regulates the expression of both miR-1247 and STMN1, and inhibition of DNA methylation could help in upregulating the expression of miR-1247 in the cells and tissues linked with NSCLC; thereby, significantly inhibiting the invasion and cell migration (Zhang et al., 2016).

Recent researches have determined the transformed expression of microRNAs and DNA methylation in the development of tumours. For instance, hypermethylation of miR-1247-5p gene is linked with the development of HCC. Researchers have additionally published the significant downregulation of microRNA 1247-5p in the cells and specimens associated with HCC. on the contrary; they ascertained that ectopic upregulation of the expression of microRNA 1247-5p substantially represses aggression and proliferation of HepG2 cells (which are liver cancer cells in human beings), induces cell apoptosis, and suppresses the growth of tumours. In this regard, upregulation of the expression of miR-1247-5p influenced 3' UTR of Wnt3 gene and emerged in the downregulation of its manifestation. The treatment with 5-Aza, which is a demethylation drug, might enormously enhance the expression of miR-1247-5p that eventually works as a tumour suppressor (Chu et al., 2017).

Studies have shown that microRNAs have a significant function in the pathogenesis of neuroblastoma. Wu et al. (2018) described that miR-1247 is downregulated within the tissues and cells related to neuroblastoma. They additionally stated that the expression of miR-1247 could remarkably suppress the propagation of cells, and induce cell cycle G0/G1 phase arrest along with cell apoptosis of neuroblastoma cells. In this regard, increase the level of miR-1247 downregulates the expression of ZNF346, which belongs to the class of Cys-2–His-2 zinc finger proteins commonly found in the brain. Researchers further noted that miR-1247 targeted 3'UTR of ZNF346 and decreased its expression; thereby, treating childhood neuroblastoma (Wu et al., 2018).

MicroRNAs, for instance, miR-27a, miR-143, miR-150, and miR-1247, have been found to have a critical task in the generation of bladder carcinoma. For instance, as with many other cancers, the expression of miR-1247 is considerably downregulated in bladder malignancy. Zhu et al., (2018) found that microRNA 1247 targeted 3'UTR of RAB36, which is a modulator of cells' growth and their development, and decreased its

expression. By targeting RAB36, miR-1247 also alters PI3K/AKT pathway, which upgrades cell continuation and cell growth, and APPL1-related epidermal factor; thereby, facilitating the activity against bladder cancer (Zhu et al., 2018).

The expression of microRNA-1247 is decreased in osteosarcoma malignant stem cells. It also affects the human osteosarcoma oncogenesis by regulating MAP3K9. MicroRNA 1247 is also downregulated in squamous carcinoma cancer tissue and lung adenocarcinoma than the healthy tissues (Zhang, J. et al., 2016). MiR-1247 has also been found to have a role in prostate cancer. In advanced stages, it becomes castration-resistant prostate cancer (CRPC), that is deadly. MiR-1247–5p interacts with 3'UTR of MYCBP2 results in its reduction in prostate cancer. It can also slightly reduces SOX9 mRNA in prostate cancer cell lines (Scaravilli et al., 2015).

2.5.6. MicroRNA as biomarkers

Along with the value of microRNAs, they were proposed as biological markers in different types of cancer. Some studies are still unproved while other is well established. miRNA can be released from their produced cells, and they are stable in blood and tissue, make it possible to detect them in samples that have been stored even after more extended periods (Meng et al., 2013). There are readily available methods for detection and quantification of miRNA. Moreover, miRNAs are tissue-specific (Landgraf et al., 2007; Shenoy & Blelloch, 2014), and have been reported that miRNA has a role in all aspects of cancer (Berindan-Neagoe et al., 2014; Pichler & Calin, 2015). Several ways perform classification of the clinical biomarkers (Emanuelli & Angelini, 2015), for instance, intracellular and extracellular based on how it measured; further classification of extracellular biomarkers into their level of invasiveness, ranging from invasive, minimally invasive, or non-invasive.

As an example of non-invasive extracellular biomarkers are those biomarkers which are extracted from blood and urine samples. Prostate-Specific Antigen (PSA), which applied as an indicator of prostate carcinoma, is a non-invasive extracellular biomarker (Ravery, V., 1999). Meanwhile, estrogen and hormone receptor levels (Yang., 2014) & mutations in BRCA gene 1/2 (Drooger., 2015), are breast cancer biomarkers, but

require invasive sample acquiring from the tissue. Facilitating the sampling technique and non-invasiveness are vital characteristics of suitable biomarkers. The fact that the cells capable of secreting miRNA into the bloodstream and The stability in of miRNAs in circulation, making them potentially well suited as biomarkers. However, as is addressed by Flatmark et al. 2016, challenges still thrive. One clinical study, after identifying six miRNAs in colorectal cancer, which considered promising, and researching their expression level in a gathering of 200 cases at stage I-III, they found few correspondences among the expression level of miRNA and the pathological state of subjects (Schee et al., 2010; 2013). The same research by Shee et al., (2013) claimed that colorectal cancers have a definite microRNA expression chart of healthy colon tissue, although the results where promising there is no miRNA colorectal cancer biomarker approved to be used clinically in colorectal cancer.

Flatmark et al. (2016) show several possible explanations. The miRNAs engagement in the tumour was discovered in 2002, and their biology is still being unrevealed, it is hardly surprising that progress has been less than initially hoped. However, several compounding issues complicate the matter. For instance, although miRNA expression can distinguish tissue types, not all miRNAs are necessarily tissue-specific. The expression of miRNA is considerable overlapping between tissues, with moderate changes for some miRNA.

A miRNA may target sites on multiple genes, and action of a miRNA in one type of tissue may utterly different in another tissue, based on the circumstances; this is essentially the presence of how many putative targets in the light of miRNA molecules. The biology of miRNAs is therefore highly complex. To be a valid biomarker, however, moderate fold changes are not enough. There must be a substantial fold change between the disease and the normal state, and this variance must be consistent. Most of the miRNA literature has contradictory results: the same miRNAs in the same tissue might be claimed as upregulated and downregulated by different studies. It is likely due to the use of different experimental detection techniques. The qRT-PCR and Microarray are more reliable gradual correct annotation in the used reference database. Research on miRNA currently suffers due to miRNAs misannotated. Efforts are, however, being made to standardise small RNA workflows to provide more reliable biomarker signatures.

3. MATERIALS AND METHODS

3.1. Study design and samples

The designed objective of this research is to study the level of six miRNAs in the serum obtained from colorectal patients and control. Dr Mustafa Duman, Kartal hospital provide the samples. We performed all the experiments in Prof. Dr Sadrettin Pence at Istanbul university/ molecular medicine department. We received an approval for clinic research from ethic board of Istanbul University, Faculty Of Medicine, under the number (481) in 23/ 03/ 2018.

The samples were obtained from Adult Turkish patients, the total number of the samples was supposed to be 80 samples; 40 samples from patients and 40 samples from the healthy individual as control. The samples will be obtained from colorectal cancer patients according to the following criteria: Newly diagnosed as a colorectal cancer patient. He/she is diagnosed with laboratory and pathological diagnostic methods. Patients should not be treated; there is no other disease and does not have a genetic predisposition. The control group should be healthy without the disease. Dr Mustafa Duman collected the samples with patient's consents; the samples were stored in EDTA tubes at -80C.

3.2. Chemical Materials

MiRNeasy Serum/Plasma Kit from Qiagen Cat No./ID: 217184.

has-miR-195 Real-time RT-PCR detection and U6 calibration kit, cohesion biosciences catalogue CPK2133.

hsa-miR-224 Real-time RT-PCR detection kit, cohesion biosciences catalogue CPK1163.

hsa-miR-126-3p Real-time RT-PCR detection kit, cohesion biosciences catalogue CPK1083.

hsa-miR-92a Real-time RT-PCR detection kit, cohesion biosciences catalogue CPK1059.

hsa-miR-214 Real-time RT-PCR detection kit, cohesion biosciences catalogue CPK1153.

hsa-miR-1247 Real-time RT-PCR detection kit, cohesion biosciences catalogue CPK.

RNA free Water (QIAGEN, cat. No 129112).

Chloroform (Merck cat. No:102445,2500).

Ethanol 99% (Merck cat. No:100983,2500).

Isopropanol 100% (Merck cat. No:100995,2500).

3.3. Consumable materials

PPAXgen blood RNA tube (Qiagen, cat. No: 762165).

Pipette tips (0-10 µl, 20-100 µl, 100-1000 µl) (AXYAGEN).

1.5 ml tubes.

2 ml tubes.

Gloves.

3.4. Types of equipment

Deep freezer (-80 °C) (Esco Lexicon II ULT Freezer).

Deep freezer (-20 °C) (Profilo).

Vortex (VWR International).

Cold centrifuge (Beckman Coulter Allegra X-30R).

Desktop Mini centrifuge (Beckman Coulter Allegra 16).

PCR machine (BioRad T100 Thermal Cycle).

Real-Time PCR Machine (Bio-Rad CFX connect TM Real-Time PCR).

Autoclave (DAIHAN SCIENTIFIC).

Spectrophotometer (Thermo Scientific NanoDrop, 2000).

Distil Water machine (ELGA Millipore).

Hepa filter Laminar Flow cabin (Thermo Scientific).

Micropipette (0-10 μ l, 20-100 μ l, 100-1000 μ l) (AXYAGEN).

Ice making machine (H-IMS-50).

3.5. Experiments

3.5.1. RNA isolation

We have used miRNeasy Serum/Plasma Kit from Qiagen Cat No./ID: 217184 The protocol has been followed. Briefly, we thawed the frozen samples, adding five volumes of QIAzol lysis buffer, which was 1000 μ l that is because we have used 200 μ l of serum. The samples were vortex and mixed well. The tubes containing the lysate were placed at room temperature for 5 minutes. After that, 3.5 μ l miRNeasy Serum/Plasma Spike-In Control and mixed thoroughly. Chloroform (200 μ l) was added then the tubes vortex for 15s. The tubes were left for 2 min at room temperature, then centrifuged at 12,000 x g at 4°C for 15 min. after centrifugation, the aqueous phase was transferred to a new collection tube. 1.5 ml 100% ethanol was added and mixed thoroughly using the pipetting procedure.

Importantly, without any delay we continued with next step, 700 μ l of the sample was transferred to RNeasy MinElute spin column fitted in a 2 ml collection tube after that centrifuged at ≥ 8000 x g at lab temperature for 15 s. Followed by discarding the pass-through and then repeated the step with the remaining sample and discard the flow-through. 700 μ l Buffer RWT was added to the RNeasy MinElute spin column and centrifuged for 15 s at ≥ 8000 x g, and the pass-through was discarded. 500 μ l Buffer RPE was pipette up onto the RNeasy MinElute spin column then centrifuged at ≥ 8000 x g for 15 s; the flow-through was discarded. 500 μ l of 80% ethanol was added to the RNeasy MinElute spin column and centrifuged for 2 min at ≥ 8000 x g. At this step, the collection tubes with the flow-through were discarded. We placed the RNeasy MinElute spin column into a new 2 ml collection tube and centrifuged for 5 minutes at full speed to dry the membrane; then we discarded the collection tube with the pass-through. Then placed

RNeasy MinElute spin column in a new 1.5 ml collection tube with adding 14 μ l RNase-free water directly to the centre of the spin column membrane and centrifuged at full speed for 1 min for RNA elution. The quantity and purity of RNA samples were tested using Nanodrop. Lastly, the RNA samples eventually stored in 1.5 ml collection tubes at -80C.

3.5.2. cDNA synthesis

We used specific Real-time RT-PCR detection kit for each miRNA (has-miR-195, hsa-miR-224, hsa-miR-126-3p, hsa-miR-92a, hsa-miR-214 and hsa-miR-1247) which are obtained from cohesion biosciences. U6 calibration kit was supplied with the has-miR-195 kit. The miRNA standard is supplied as 1pmol dry powder oligo stock. The tube containing the powder is centrifuged at 10000 rpm/min for 1 minute, 1 ml RNA dilution buffer was added, to dilute the stock to 1 nM. Then in another tube to 0.1nM, five serial dilutions were performed.

3.5.2.1. Prepare the miRNA RT primer

The supply of the miRNA primer is as 10 μ M; it diluted to 1 μ M. in the case of has-miR-195 Real-time RT-PCR detection and U6 calibration kit, primer mix is prepared by adding 12 μ l of miR-195 and 12 μ l U6 primer, then adding 96 μ l Rnase free H₂O. Then the primer mix was stored at -20 °C.

3.5.2.2. The RT reaction mix

Table 3 1: RT reaction mix

Component	Vol/1 rxns
5 X Master Mix	4 μ l
dNTP (10mM)	0.75 μ l
MiR-RT primers/ primer mix (1 μ M)	1.2 μ l
MMLV reverse transcriptase (200 U/ μ l)	0.2 μ l
RNA sample	3 μ l
RNase free H ₂ O	Up to 20 μ l

The reagents except RNA sample were mixed in the tube using volumes regarding the numbers of samples. Then 17 μl is pipette in tubes according to sample number, and then 3 μl of RNA from each sample is added to the mix. In addition to that, five tubes were for the standard solutions.

3.5.2.3. MiRNA RT reaction

Although the RT reaction for each miRNA performed separately, the protocol is the same. The tubes are arranged in RT-PCR thermal cycle instrument, using the standard RT reaction program according to the protocol. The cDNA product samples then stored at $-4\text{ }^{\circ}\text{C}$.

Table 3-1: RT thermal cycles

25 $^{\circ}\text{C}$	30 minutes
42 $^{\circ}\text{C}$	30 minutes
84 $^{\circ}\text{C}$	5 minutes

3.5.3. miRNAs Real-time PCR reaction.

3.5.3.1. Handling the miRNA RT product

For 20 μl reaction size, 2 μl cDNA, the miRNA RT reaction product is used as the template for the real-time PCR step.

3.5.3.2. Preparing a reaction mix for Real-Time PCR reaction

Table 3-2: Reaction mix for Real-Time PCR reaction of miRNA

Component	Vol/ 1rxns
2X Real-Time Master Mix	10 μl
MiR specific primer set (10nM)	0.4 μl
ROX reference dye 5X	0.4 μl
PCR enhancer	1.5 μl
Taq DNA polymerase (5U/ μl)	0.2 μl
MiRNA RT product	2 μl
dd H ₂ O	Up to 20 μl

For U6:

Table 3-3: Reaction mix for Real-Time PCR reaction of U6

Component	Vol/ 1rxns
2X Real-Time Master Mix	10 µl
U6 specific primer set (10nM)	0.4 µl
ROX reference dye 5X	0.4 µl
PCR enhancer	1.5 µl
Taq DNA polymerase (5U/ µl)	0.2 µl
MiRNA RT product	2 µl
dd H ₂ O	Up to 20 µl

3.5.3.3. MiRNAs Real-Time PCR reaction performing

The program in Real-Time PCR is settled up as the following, the second and third step repeated for 40 cycles. SYBR is for detection and ROX for reference dye, and the detection step is at 62 °C.

Table 3-4: Real-Time PCR reaction cycles

95 °C	3 minutes
95 °C	12 seconds repeats for 40 cycle
62 °C	40 seconds

3.6. Data Analysis

3.6.1. Manipulating real-time PCR result

The C_T is threshold cycle which represents the endpoint result of real-time PCR, and as described in the paper of Livak et al., (2001), if the statistical presentation by using the raw values of C_t should be avoided, because the C_t is an exponential and not a linear term. The following formulas Yuan et al., (2006) was used to interpret the C_t value resulted from the samples from patients and control.

Equation 3-1: $\Delta\Delta Ct$

$$\Delta\Delta Ct = (Ct_{\text{Target}} - Ct_{\text{U6}})_{\text{Cancer}} - (Ct_{\text{Target}} - Ct_{\text{U6}})_{\text{Control}} \cdot (\text{Livak et al., 2001})$$

Equation 3-2: ΔCt

$$Ratio = \frac{(E_{\text{target}})^{\Delta Ct_{\text{target}}}}{(E_{\text{reference}})^{\Delta Ct_{\text{reference}}}}$$

$$\Delta Ct_{\text{target}} = Ct_{\text{control}} - Ct_{\text{cancer}}$$

$$\Delta Ct_{\text{reference}} = Ct_{\text{control}} - Ct_{\text{reference}}$$

Equation 3-3: $2^{-\Delta\Delta Ct}$

$$Ratio = 2^{-\Delta\Delta Ct}$$

$$\Delta\Delta Ct = \Delta Ct_{\text{reference}} - \Delta Ct_{\text{target}}$$

4. RESULTS

4.1. RNA isolation and cDNA synthesis

RNA is separated efficiently from all the blood samples of the patients and the control. cDNA synthesised from the isolated RNA; the average of all samples was between 400-500 $\mu\text{g/ml}$. The number of samples was 40 samples obtained from control volunteers, 40 samples obtained from colorectal cancer patients (11 were female, and 29 were males); the age was ranging from 24 years to 87 years old. All the data is contained in raw data section.

Firstly, as we can notice from the database that, number of male patients is about two times more than female patients, moreover the age of the patients is different between males and females; females patients are having the disease at an older age while among our male –patients there are patients in their 20s and 30s.

4.2. Real-Time PCR

4.2.1. Mir-195 is downregulated among colorectal cancer patients

Firstly, as we can notice from the database that, number of male patients is about two times more than female patients, moreover the age of the patients is different between males and females; females patients are having the disease at an older age while among our male –patients there are patients in their 20s and 30s.

The table shows the raw data which collected from the quantification measurements of miR-195 and U6, as a housekeeping gene, together with the calculation of the ratio, is in raw data section. Equations (Equation, 3-1) and (Equation 3-2) were used to calculate $\Delta\Delta\text{ct}$ and $2^{-\Delta\Delta\text{ct}}$. Moreover, the data was plotted using Log, to avoid skewness.

Before applying analytical tests to identify the significance of the result, we can notice that miR-195 is lower among the patients' group. By normalising the ratio between the average of the ration of patients and those of the control group and calculate the Log

value; the ratio which means fold change is (- 18). That indicates that miR-195 among the patient group is about five times lower than the healthy group.

By using the one-way ANOVA test, we are comparing the means of the groups; the significance will be considered if the p-value is equal to or lower than 0.05. The following tables and graphs represent the data obtained by using the SPSS (version 22) for the statistical analysis of the data.

Table 4-1: The result of descriptive analysis using SPSS.22

Descriptive Comparison of MiR195 Expression								
	N	Mean	Std. Dev.	Std. Error	95% Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
Control	36	0.26	4.74	0.79	-1.35	1.86	-6.74	8.47
Stage II	16	-2.49	2.53	0.63	-3.84	-1.14	-9.56	0.69
Stage III	13	-4.94	1.53	0.43	-5.86	-4.01	-8.99	-2.50
stage IV	7	-8.49	2.88	1.09	-11.15	-5.82	-12.3	-4.76
Total	72	-2.14	4.68	0.55	-3.24	-1.04	-12.3	8.47

The data are plotted in the following line chart (Figure 4-1), shows that the means of the expression level of microRNA-195 among control group and malignancy stage (II, III and IV). The difference in expression level is apparent with the lowest expression level among stage IV colorectal cancer patients. The data are plotted in the following line chart (Figure 4-1), shows that the means of the expression level of microRNA-195 among control group and malignancy stage (II, III and IV). The difference in expression level is apparent with the lowest expression level among stage IV colorectal cancer patients.

Table 4-2: The one-way ANOVA test based on cancer and control includes mean and the significance level of the expression level of miR-195.

One-way ANOVA of MiR195 Expression					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	592.552	3	197.517	13.964	0.00003
Within Groups	961.831	68	14.145		
Total	1554.383	71			

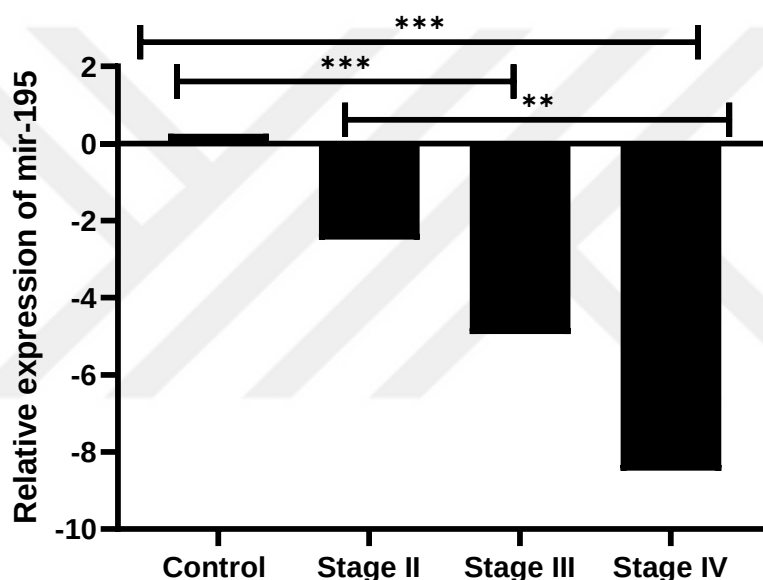


Figure 4-1: Line graph, representing the values of the mean of various groups of the studies (control, stage II, stage III & stage IV cancer patients), indicating the standard error and significance value; * if P-value ≤ 0.05 ; ** if P-value ≤ 0.01 and * if P-value ≤ 0.001**

As we can notice from this graph, the mean is significantly different among those groups. The highest value is within the control group, while the value is decreasing to reach its lower value among patients with TNM stage IV colorectal cancer. Also, the table (Table 4-2) shows the data obtained using one-way ANOVA, as it is evident that P-value is less than 0.05, which indicates that the variation between groups is significant. It could

be concluded from the information in the table (4-3) that a significant variation is found between the cancer patients with stage III & IV and the control group, while there is an insignificant difference between the stage II with the control, and between stage III & IV.

Using the Pearson correlation test to examine the correlation within the subjects with CRC and the control group indicates a significant value among them, as it is shown in tables (4-4 & 4-5). However, there no significant correlation or difference between the males and females and the smoker with non-smoker as we can see in (Table 4-6) and (Table 4-7), respectively; the P-value is more than 0.05, which indicate insignificant value.

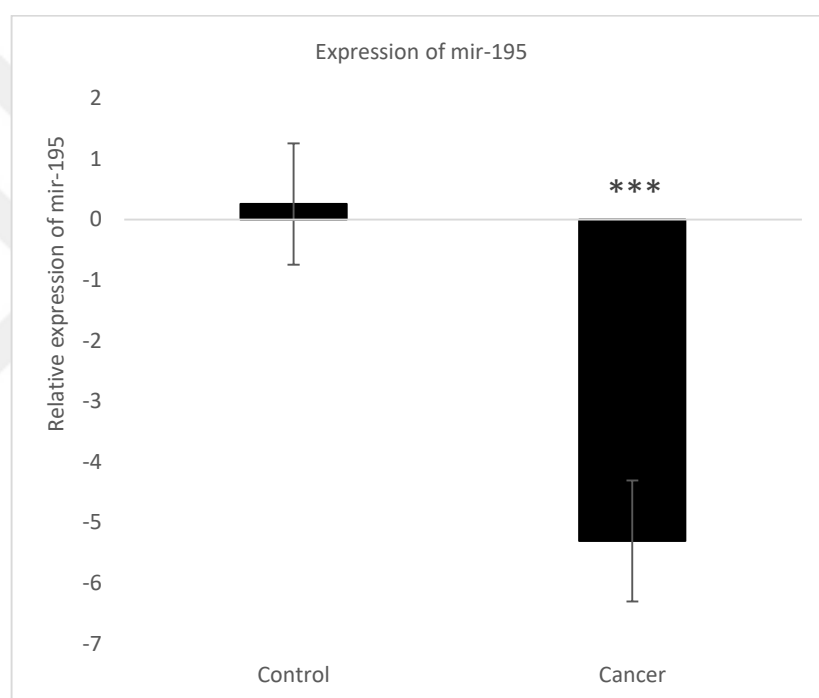


Figure 4-2: Relative expression of miR-195 among cancer samples and control

Table 4-3: The data obtained from multiple comparisons of miR-195 expression level between the control group and various cancer stages

Multiple Comparisons miR195 Expression among control and cancer stages using Pearson correlation						
Groups		Mean	Std.	Sig.	95% Confidence Interval	
		Difference (I-J)	Error		Lower Bound	Upper Bound
Control	Stage II	2.75	1.13	0.08	-0.23	5.73
	Stage III	5.19*	1.22	0.00	1.99	8.40
	Stage IV	8.74*	1.55	0.00	4.65	12.83
Stage II	Control	-2.75	1.13	0.08	-5.73	0.23
	Stage III	2.45	1.40	0.31	-1.25	6.14
	Stage IV	5.99*	1.70	0.00	1.51	10.48
Stage III	Control	-5.19*	1.22	0.00	-8.40	-1.99
	Stage II	-2.45	1.40	0.31	-6.14	1.25
	Stage IV	3.55	1.76	0.19	-1.10	8.19
Stage IV	Control	-8.74*	1.55	0.00	-12.83	-4.65
	Stage II	-5.99*	1.70	0.00	-10.48	-1.51
	Stage III	-3.55	1.76	0.19	-8.19	1.10

*. The mean difference is significant at the 0.05 level.

Table 4-4: The Pearson correlation test of miR-195 data shows the Pearson correlation and significance

Pearson Correlations miR195 Expression level			
		Control	patient
Control	Pearson Correlation	1	-.329*
	Sig. (2-tailed)		0.050
	N	36	36
patient	Pearson Correlation	-.329*	1
	Sig. (2-tailed)	0.050	
	N	36	36

*. Correlation is significant at the 0.05 level (2-tailed).

Table 4-5: One-way ANOVA based on gender in regards to the expression level of miR-195

One-Way ANOVA based on gender					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.652	1	0.652	0.063	0.803
Within Groups	351.456	34	10.337		
Total	352.108	35			

Table 4-6: One-way ANOVA data representing the comparison of mean between smoker and non-smoker in regards to the expression level of miR-195

One-Way ANOVA based on smoking					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.069	1	1.069	0.104	0.750
Within Groups	351.039	34	10.325		
Total	352.108	35			

Table 4-7: One-way ANOVA data representing the comparison of mean between age groups in regards to the expression level of miR-195

One-Way ANOVA based on Age					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	46.180	3	15.393	1.450	.250
Within Groups	286.707	27	10.619		
Total	332.887	30			

Figure (4-2) shows the Log value of $2^{-\Delta\Delta Ct}$ of both groups (Cancer group and Control group), it indicates the differences among these groups with a significance value 0.05 as reported in the table (4-2). Here we can see that the patient's group has a lower level of serum microRNA-195 expression level than the control group.

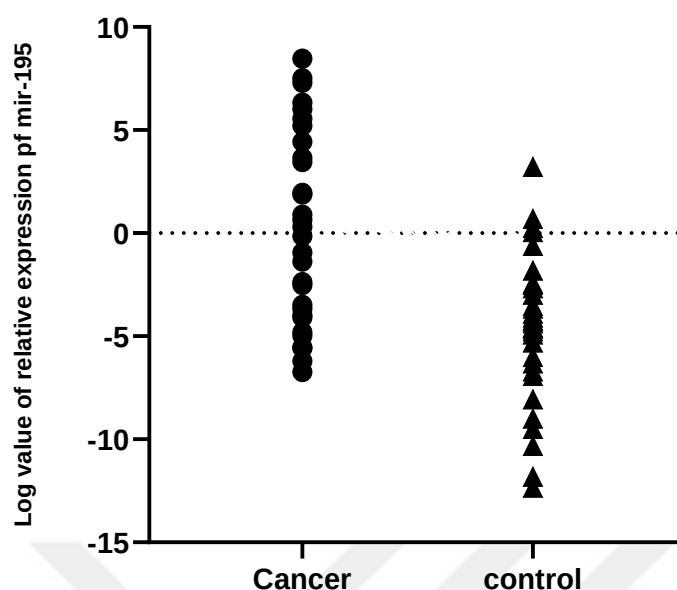


Figure 4-3: The Log value of $2^{-\Delta\Delta Ct}$ of the expression level of miR-195 among the cancer patients and the control group, illustrating the data with standard error

4.2.2. MiR-214 is upregulated in the serum samples of colorectal patients

The result shows that according to the analytical analysis of the collected data from the quantification measurements of miR-214. The data is illustrated in raw data section along with the ratio and fold change calculation. Equation 3-1; 3-2 and 3-3 were used to calculate $\Delta\Delta Ct$ and $2^{-\Delta\Delta Ct}$. The data is plotted using Log in order to avoid skewness.

By the aid of SPSS.22 program to analyse the data statistically using one-way ANOVA, Pearson correlation and descriptive data, the data could be concluded with an assumption that miR-214 is upregulated with a fold change up to 6.6 times; the patients with cancer have serum level of miR-214 higher than those of control individuals by more than two-fold.

Figure (4-3) plots the mean of the expression level of miR-214, indicating the difference between the control group and the patients with different colorectal malignancy grades; stage II, stage III and stage IV. As we can see, miR-214 is upregulated

in CRC in comparison with the control group, as we can notice, the expression level is also increasing with the cancer progression. Table (4-8) is concerning of the Pearson correlation test, correlating the data of expression level of miR-214, particularly the mean value, in between the control group and colorectal cancer stage” II, III and IV” patients. It could be seen from this result that there is an apparent consequential divergence amongst the control and three stages of the disease with a significant value less than 0.05. The stage II colorectal cancer has a significant difference between it and the control group with a value less than 0.05, while there is no significant value in regarding the correlation of stage II with stage III and stage IV colorectal cancer. Similarly, stage III and stage IV have a significant correlation of only the control group. It could be concluded that the correlation is only significant when comparing the control group with the colorectal cancer stages and no significant comparison between the stages.

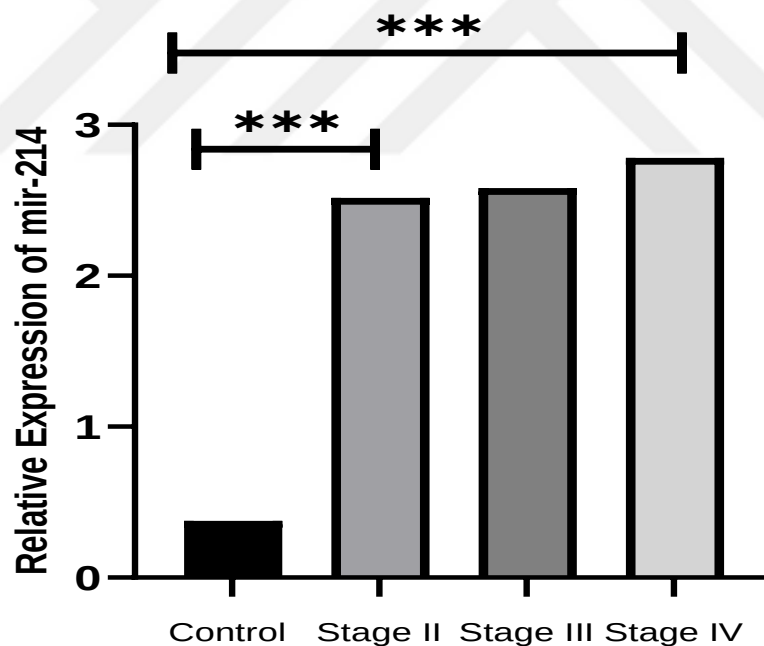


Figure 4-4: A graph, showing the data (mean value) of the data obtained from the descriptive analysis of expression level data of miR-214 among the control group and three different colorectal cancer stages

Table 4-8: The Pearson correlation test, to study the relationship between the miR-214 expression level of different cancer stages, and compare each group with the other three groups

Multiple Comparisons of miR-214						
Dependent Variable:						
Tukey HSD						
Groups		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
		(I-J)			Lower Bound	Upper Bound
Control	Stage II	-2.14132412*	0.25	0.000	-2.8000919	-1.4825563
	Stage III	-2.20546249*	0.26	0.000	-2.8987956	-1.5121294
	Stage IV	-2.40538087*	0.34	0.000	-3.2892106	-1.5215511
Stage II	Control	2.14132412*	0.25	0.000	1.4825563	2.8000919
	Stage III	-0.06	0.31	0.997	-0.8730270	0.7447503
	Stage IV	-0.26	0.37	0.892	-1.2411673	0.7130538
Stage III	Control	2.20546249*	0.26	0.000	1.5121294	2.8987956
	Stage II	0.06	0.31	0.997	-0.7447503	0.8730270
	Stage IV	-0.20	0.38	0.952	-1.2006585	0.8008218
Stage IV	Control	2.40538087*	0.34	0.000	1.5215511	3.2892106
	Stage II	0.26	0.37	0.892	-0.7130538	1.2411673
	Stage III	0.20	0.38	0.952	-0.8008218	1.2006585

*. The mean difference is significant at the 0.05 level.

The Pearson correlation test performed using the data of patients and control to confirm the significant correlation between the control group and the colorectal cancer patients entirely without any consideration regarding of the different stages, the gender of health status; the result confirms that there is a notable distinction amidst those groups as we can see in the following table (Table 4-9).

Table 4-9: The Pearson Correlations of the mean of the serum expression level of miR-214 among patients and the control

Pearson Correlations of mean of the serum expression level of miR-214 among patients and the control			
		Control	Patient
Control	Pearson Correlation	1	.675**
	Sig. (2-tailed)		0.000
	N	35	35
Patient	Pearson Correlation	.675**	1
	Sig. (2-tailed)	0.000	
	N	35	41

**. Correlation is significant at the 0.01 level (2-tailed).

When comparing the mean of the miR-214 expression level among the colorectal patients, there is, who are smoker and non-smoker patients; there is insignificant value. There is an insignificant effect of smoking on the expression level of miR-214 in those patients. The data of the one-way ANOVA test based on smoking is plotted in the following table (Table 4-10).

Table 4-10: One-way ANOVA test results, showing the comparison in the mean of the expression level data of miR-214 among the smoker and non-smoker patients

MiR-214 Expression One-Way ANOVA based on smoking					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.002	1	0.002	0.069	0.795
Within Groups	1.096	33	0.033		
Total	1.098	34			

Similarly, the difference in the effect of the gender on the circulating level of expression of miR-214 in the cases with colorectal cancer is insignificant; the significance value is more than 0.05, we can notice from the table (4-11), that the significance value is 0.322.

Table 4-11: One-way ANOVA test results, showing the comparison in the mean of the expression level data of miR-214 among the male and female patients

One way ANOVA based on gender					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.033	1	0.033	1.011	0.322
Within Groups	1.066	33	0.032		
Total	1.098	34			

Table 4-12: One-way ANOVA test results, showing the comparison in the mean of the expression level data of miR-214 among different age groups

One-Way ANOVA based on age					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.050	3	.017	1.608	.211
Within Groups	.282	27	.010		
Total	.333	30			

Lastly, plotting the Log value of $2^{-\Delta\Delta C_t}$ in the following bar and whisker chart, indicating the significant difference among the control and the patient group. As it is noticeable from the graph, that the patient group serum level of miR-214 expression is higher than the control group.

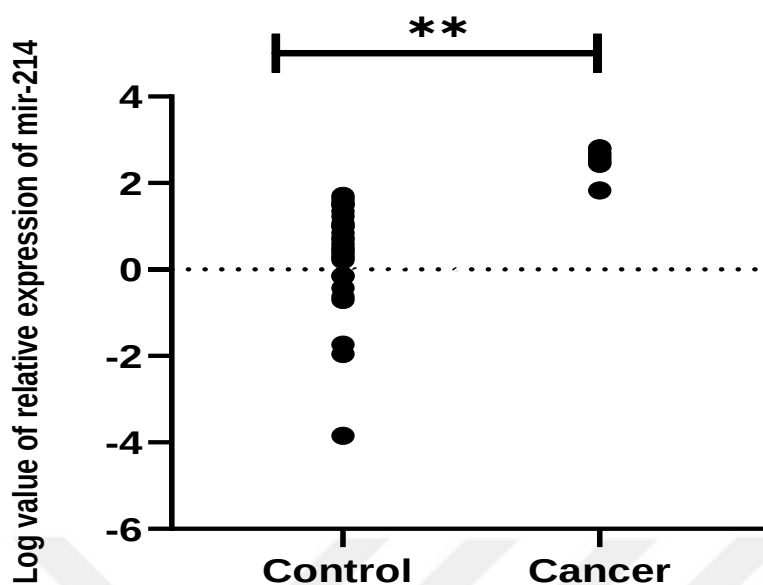


Figure 4-5: Bar and whisker chart showing the Log value of $2^{-\Delta\Delta Ct}$ of the serum expression level of miR-214 in colorectal cancer patients and control

4.2.3. MiR-224 is downregulated in the serum of colorectal cancer patients

The same process of data collection and calculation is repeated to the data obtained from qRT-PCR which executed to study the expression level of miR-224 among control, and patients with CRC. The data is summarised in the table in raw data section as it contains the values of Ct, ΔCt , $\Delta\Delta Ct$, $2^{-\Delta\Delta Ct}$ and logarithmic value of patients and the control group.

The first test was performed is one-way ANOVA, aiming to contrast the mean of the data concerning the expression level of microRNA 224 among patients and the control, showing that the significance value is lower than 0.05 indicating the significance of these result. Plotting the data (Mean) of miR-224 expression level among the groups as a line chart in figure (4-5), it is noticed that the expression level of microRNA 224 is decreasing with the development of CRC and with stage progress. The highest value is within the control group, and the lowest is within stage IV CRC patients.

Table 4-13: One-way ANOVA test of miR-224 expression level between the data of control and the patients

One-way ANOVA of miR-224 Expression, based on cancer stages and control					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1075.317	3	358.439	19.454	0.000
Within Groups	1050.201	57	18.425		
Total	2125.519	60			

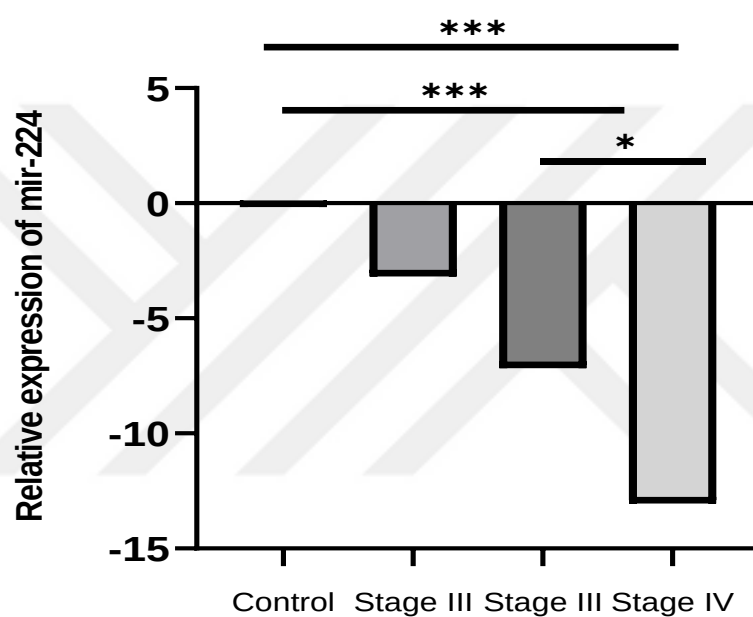


Figure 4-6: Line chart plotting the data of one-way ANOVA of the mean of different stages of colorectal cancer and the control group

Table 4-14: Pearson Correlations between patients and control. It shows the result of Pearson correlation test among control and different stages of CRC, comparing the serum expression level of miR-224

Multiple Comparisons of miR-224 expression among different stages of CRC and control						
Dependent Variable:						
Tukey HSD						
Groups		Mean	Std.	Sig.	95% Confidence Interval	
		Difference (I-J)	Error		Lower Bound	Upper Bound
Control	Stage II	3.28	1.38	0.09	-0.37	6.94
	Stage III	7.26029010*	1.56	0.00	3.13	11.39
	Stage IV	13.14149678*	1.91	0.00	8.07	18.21
Stage II	Control	-3.28	1.38	0.09	-6.94	0.37
	Stage III	3.98	1.78	0.13	-0.73	8.68
	Stage IV	9.85742271*	2.09	0.00	4.31	15.40
Stage III	Control	-7.26029010*	1.56	0.00	-11.39	-3.13
	Stage II	-3.98	1.78	0.13	-8.68	0.73
	Stage IV	5.88120668*	2.22	0.05	0.02	11.75
Stage IV	Control	-13.14149678*	1.91	0.00	-18.21	-8.07
	Stage II	-9.85742271*	2.09	0.00	-15.40	-4.31
	Stage III	-5.88120668*	2.22	0.05	-11.75	-0.02

*, The mean difference is significant at the 0.05 level.

By performing Pearson correlation test in SPSS.22 (Table 4-14), indicating that the expression level of microRNA 224 among CRC patients with stage 4 disease is correlating significantly with other groups control, stage two CRC and stage three CRC. While the control expression level of miR-224 is only significantly differed from stage III and IV and no significant correlation with stage II. That indicates that the serum expression level of microRNA 224 is more decreased when the disease progress and metastasise. The correlation between the control and the CRC patient as a whole was evaluated using the Pearson correlation test, as presented in the table (4-15). However, there is no overall significant correlation between the patients and the control group.

Table 4-15: Pearson Correlations between patients and control. It shows the result of Pearson correlation test among control and different stages of CRC, comparing the serum expression level of miR-224

Pearson Correlations between patients and control			
		Control	patient
Control	Pearson Correlation	1	0.242
	Sig. (2-tailed)		0.189
	N	31	31
patient	Pearson Correlation	0.242	1
	Sig. (2-tailed)	0.189	
	N	31	31

Amongst the fraction of CRC cases, there are smoker and non-smoker; males and females as it is aforementioned. One-way ANOVA test was conducted, comparing the mean of their data to assess if there is a notable effect of smoking and sex on the serum expression level of miR-224. The result reveals no significant effect of the smoking, gender and age on the expression level of this micro-RNA, as represented in (Table 4-16; 4-17; and 4-18). However, the significance value is slightly higher than 0.05 concerning one-way ANOVA test based on age (table 4-18), that is may because of the differences in the number of male and female patients and different cancer stages among them.

Table 4-16: One-way ANOVA testing for significant difference for the expression level of miR-224 to the 0.05 level based on smoking

One-Way ANOVA (MiR-224 expression concerning smoking)					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	37.409	1	37.409	2.246	.145
Within Groups	483.099	29	16.659		
Total	520.508	30			

Table 4-17: One-way ANOVA testing for significant difference for the expression level of miR-224 to the 0.05 level based on gender

One-Way ANOVA (MiR-224 expression among different gender)					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	61.904	1	61.904	3.914	.057
Within Groups	458.604	29	15.814		
Total	520.508	30			

Table 4-18: One-way ANOVA testing for significant difference for the expression level of miR-224 to the 0.05 level based on age

One-Way ANOVA (MiR-224 expression among different age groups)					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20.272	3	6.757	.365	.779
Within Groups	500.236	27	18.527		
Total	520.508	30			

Figure (4-6) is plotting the logarithmic value of the $2^{-\Delta\Delta C_t}$ of the patients, and the control confirms that the cancer individuals have a serum concentration of miR-224 more repressed than the control group. It could be concluded that the miR-224 is reduced in cancer; moreover, its level is decreased with the advancing of the disease.

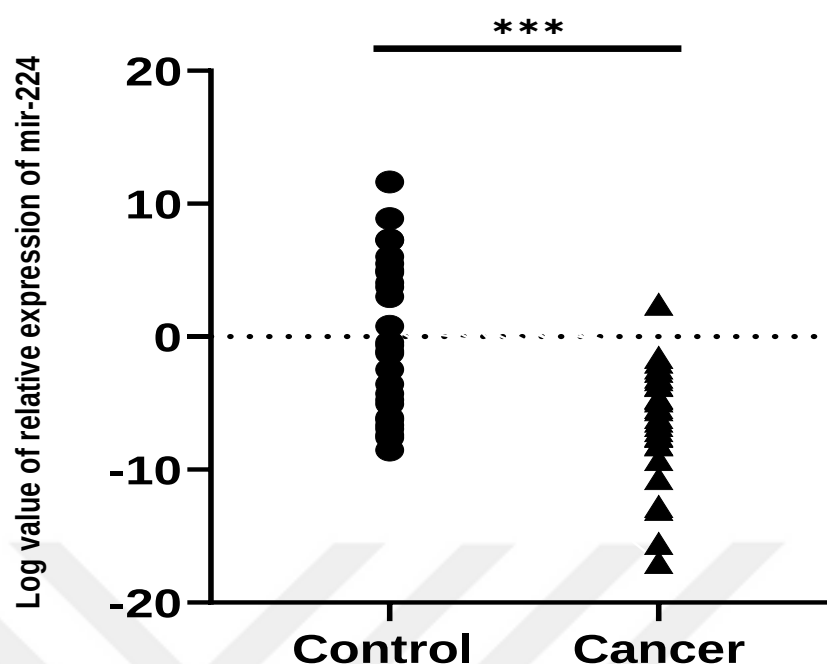


Figure 4-7: Logarithmic value of the $2^{-\Delta\Delta C_t}$ of miR-224 in the serum the patients and the control

4.2.4. MiR-126-3p is down-regulated in the serum of colorectal cancer patients

The data obtained from performing the qRT-PCR to study the mode of miR-126 expression amidst control and subjects is illustrated in the following table at raw data section. Data is summarised as it contains the values of C_t , ΔC_t , $\Delta\Delta C_t$, $2^{-\Delta\Delta C_t}$ and logarithmic value of diseased subjects and the healthy group. Primarily, calculating the ratio of alteration in the status of representation of microRNA 126, revealing that there is a net – 9 fold change between the control and patients group. The data indicates that the miR-126 cast level in the serum of CRC cases is approximately nine-fold lower than those of control. The fold change is not significant to be considered as a specific in colorectal cancer, that could not be unbeneficial as a significant difference in the diagnosis or prognosis. However, there is a distinct variation among cancer stages.

Even though there is nine-fold decrease between the patients and the control, one-way ANOVA test shows that there is no significant difference between the control group and CRC patients, as we can notice from the table (4-19) the significance value is more than 0.05.

Table 4-19: One-way ANOVA test, which performed to study the comparison of miR-126 expression level between the data of control and the patients

One-way ANOVA MiR-126 Expression					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	54.728	3	18.243	1.291	0.285
Within Groups	932.710	66	14.132		
Total	987.438	69			

Similarly, the data distinguish between the smoker and non-smoker patients, and between the male and female, indicates no notable impression of these varieties on the expression level of the disease. The data in the table (4-20) and (4-21) represents the result of the one-way ANOVA test concerning smoking and gender, respectively.

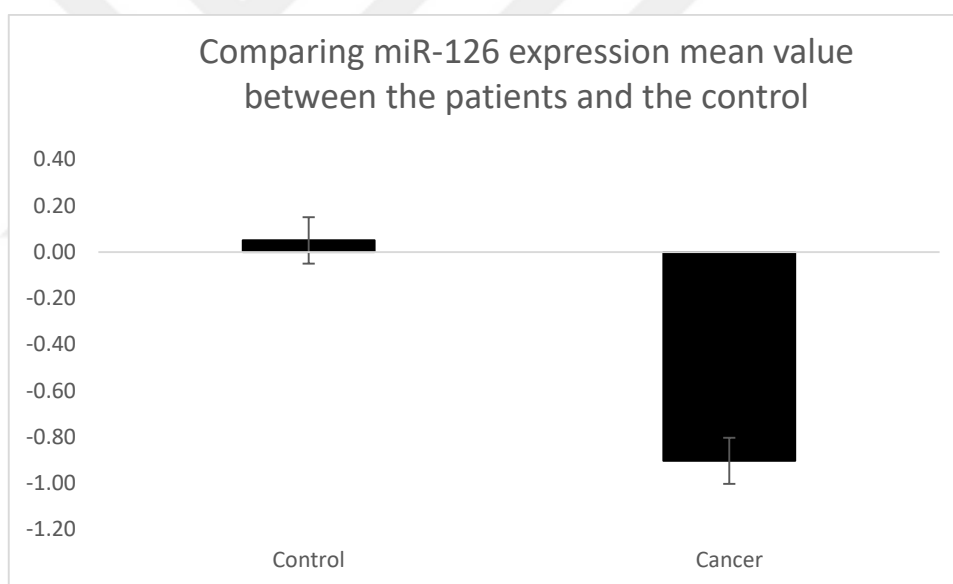
Table 4-20: One-way ANOVA test of miR-126 expression level based on smoking

One-way ANOVA testing the effect of smoking on the miR-126 expression					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.231	1	2.231	1.210	0.280
Within Groups	53.460	29	1.843		
Total	55.691	30			

Table 4-21: One-way ANOVA test of miR-126 expression level based on gender

One-way ANOVA of miR-126 expression among different gender					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.208	1	1.208	0.643	0.429
Within Groups	54.483	29	1.879		
Total	55.691	30			

The comparison between the mean value of miR-126 expression level in CRC patients and control, reveals that the control group has a higher level of miR-126 than the CRC patients. The data is plotted in the figure (4-7).

**Figure 4-8: The comparison of the mean value of miR-126 between the CRC patients and control**

The Pearson correlation test result when correlating the control and different cancer stages indicates that there is an insignificant correlation among the stages of cancer and the control as it is noticeable in the table (4-22).

Table 4-22: the Pearson correlation test of the patients and control expression level of miR-126

Pearson Correlations test of patients and control expression level of miR-126		Control	Stage II	Stage III	Stage IV
Control	Pearson Correlation	1	0.249	0.213	-0.560
	Sig. (2-tailed)		0.305	0.484	0.247
	N	40	19	13	6
Stage II	Pearson Correlation	0.249	1	-0.230	0.211
	Sig. (2-tailed)	0.305		0.450	0.688
	N	19	19	13	6
Stage III	Pearson Correlation	0.213	-0.230	1	-0.200
	Sig. (2-tailed)	0.484	0.450		0.704
	N	13	13	13	6
Stage IV	Pearson Correlation	-0.560	0.211	-0.200	1
	Sig. (2-tailed)	0.247	0.688	0.704	
	N	6	6	6	6

However, plotting the mean value in figure (4-8) indicates the difference in the expression level between the control group which has the highest level of expression of miR-126 among the groups and the lower level ranging in the CRC stages.

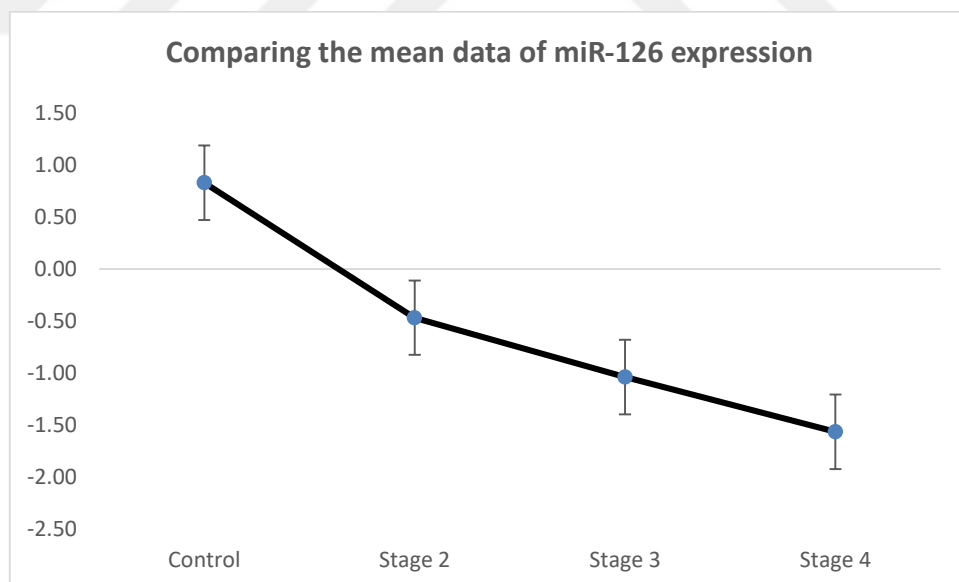
**Figure 4-9: Comparison of the mean value of miR-126 expression level among control and CRC stages**

Figure (4-9) Plotting the logarithmic value of the $2^{-\Delta\Delta Ct}$ of the patients, and the control confirms that the patients have lower serum level of miR-126 than the control group. It could be concluded that the miR-126 is downregulated in cancer; moreover, its level is decreased with the progress of the disease.

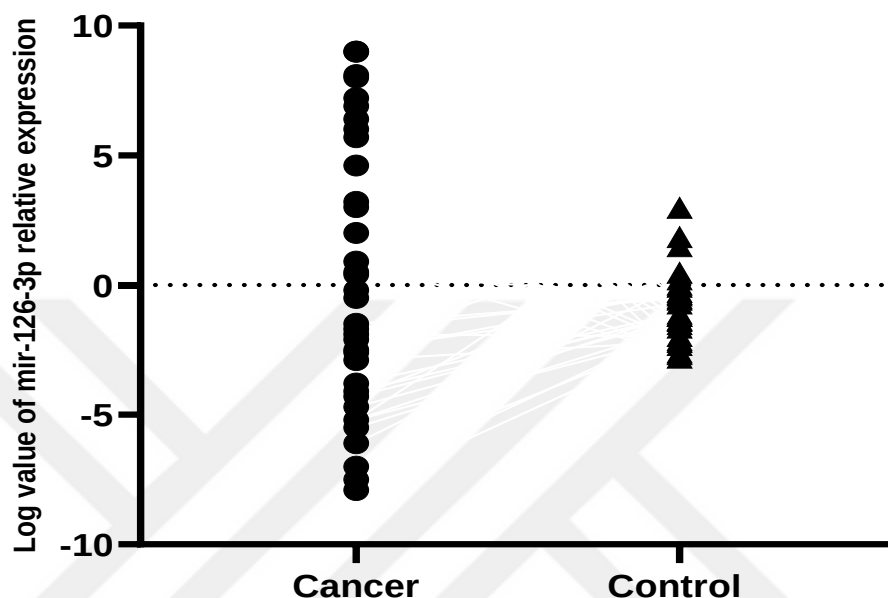


Figure 4-10: The logarithmic value of the $2^{-\Delta\Delta Ct}$ of the patients and the control

4.2.5. MiR-1247 is downregulated among CRC patients

The data obtained from the qRT-PCR which executed to study the expression level of miR-1247 among control, and CRC patients is presented among raw data table. The data is summarised, including the values of Ct, ΔCt , $\Delta\Delta Ct$, $2^{-\Delta\Delta Ct}$ and logarithmic value of patients and the control group. Primarily, calculating the ratio of change in the expression level of miR-1247, revealing that there is a net -11 fold change between the healthy and cases group. The data indicates that the miR-1247 expression level in the serum of CRC patients is eleven-fold lower than those of control.

Table 4-23: One-way ANOVA testing for significant difference for the expression level of miR-1274 to the 0.05 level based on cancer and control

One-way ANOVA					
MiR-1247 Expression					
Between Groups	174.767	3	58.256	25.883	0.001
Within Groups	155.300	69	2.251		
Total	330.066	72			

Table 4-24: The One-way ANOVA testing of the significance level of miR-1247 to 0.05 based on control and different cancer stages

Multiple Comparisons						
Dependent Variable:						
Tukey HSD						
Groups		Mean	Std. Error	Sig.	95% Confidence Interval	
		Difference			Lower Bound	Upper Bound
		(I-J)				
Control	Stage II	0.950	0.428	0.127	-0.175	2.076
	Stage III	2.99222148*	0.487	0.000	1.709	4.275
	Stage IV	4.88437498*	0.663	0.000	3.139	6.630
Stage II	Control	-0.950	0.428	0.127	-2.076	0.175
	Stage III	2.04215688*	0.540	0.002	0.620	3.464
	Stage IV	3.93431039*	0.703	0.000	2.085	5.784
Stage III	Control	-2.9922214*	0.487	0.000	-4.275	-1.709
	Stage II	-2.0421568*	0.540	0.002	-3.464	-0.620
	Stage IV	1.892	0.740	0.060	-0.057	3.842
Stage IV	Control	-4.8843749*	0.663	0.000	-6.630	-3.139
	Stage II	-3.9343103*	0.703	0.000	-5.784	-2.085
	Stage III	-1.892	0.740	0.060	-3.842	0.057

*. The mean difference is significant at the 0.05 level.

Interpreting the data in the table (4-23) clarifies that there is a meaningful distinction separating the cancer subjects and the control with a significance value 0.001, which is less than 0,05. At the same time, from the table (4-24), the CRC stages among patients significantly differ with a significance value less than 0,05. However, there is no

notable variation between the expression level of miR-1247 in CRC stage IV and stage III. Table (4-25) indicates that smoking and is significantly affected the expression level of miR-1247, with a meaningful mark 0,03. in contrary, gender and age represented an insignificant value, 0.122 & 0.48, respectively.

Table 4-25: One-way ANOVA testing for significant difference for the expression level of miR-1247 to the 0.05 level based on smoking

One-Way ANOVA of miR-1247 Expression based on smoking					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14.244	1	14.244	5.212	.030
Within Groups	79.262	29	2.733		
Total	93.507	30			

Table 4-26: One-way ANOVA testing for significant difference for the expression level of miR-1247 to the 0.05 level based on gender

One -Way ANOVA of miR-1247 Expression based on gender					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.511	1	7.511	2.533	.122
Within Groups	85.996	29	2.965		
Total	93.507	30			

Table 4-27: One-way ANOVA testing for significant difference for the expression level of miR-1247 to the 0.05 level based on Age

One -Way ANOVA of miR-1247 Expression based on Age					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.046	3	2.682	.847	.480
Within Groups	85.460	27	3.165		
Total	93.507	30			

Table 4-28: illustrate One-way ANOVA testing significance of miR-1247 to 0.05 in the control and CRC patient with different stages

		Correlations			
		Control	Stage II	Stage III	Stage IV
Control	Pearson Correlation	1	0.365	.566*	0.023
	Sig. (2-tailed)		0.125	0.044	0.966
	N	35	19	13	6
Stage II	Pearson Correlation	0.365	1	0.212	0.097
	Sig. (2-tailed)	0.125		0.487	0.855
	N	19	19	13	6
Stage III	Pearson Correlation	.566*	0.212	1	-0.745
	Sig. (2-tailed)	0.044	0.487		0.090
	N	13	13	13	6
Stage IV	Pearson Correlation	0.023	0.097	-0.745	1
	Sig. (2-tailed)	0.966	0.855	0.090	
	N	6	6	6	6

*. Correlation is significant at the 0.05 level (2-tailed).

One-way ANOVA tests the significance level of expression of miR-1247 in different cancer stages indicates that a meaningful variation has been found between the control and stage III as it is representing in the table (4-28). Figure 4-10 plots the mean value of miR-1247 expression level in CRC patients and control, is showing that the level of expression is decreasing with the disease development and progression.

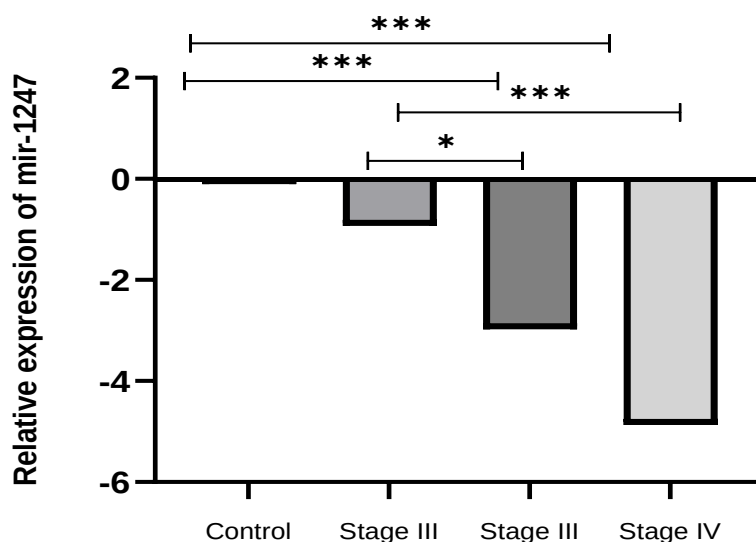


Figure 4-11: the line chart represents the mean value of the expression level of miR-1247 in CRC patients and control

Plotting mean of the circulating level of expression of miR-1247 in colorectal patients is more depressed than the expression range in the control class, indicating the downregulation of miR-1247 in blood samples from patients with colorectal cancer. Additionally, plotting the all logarithmic value of $2^{-\Delta\Delta Ct}$ of miR-1247 shows the down-regulation of miR-1247 expression level among the patient's group.

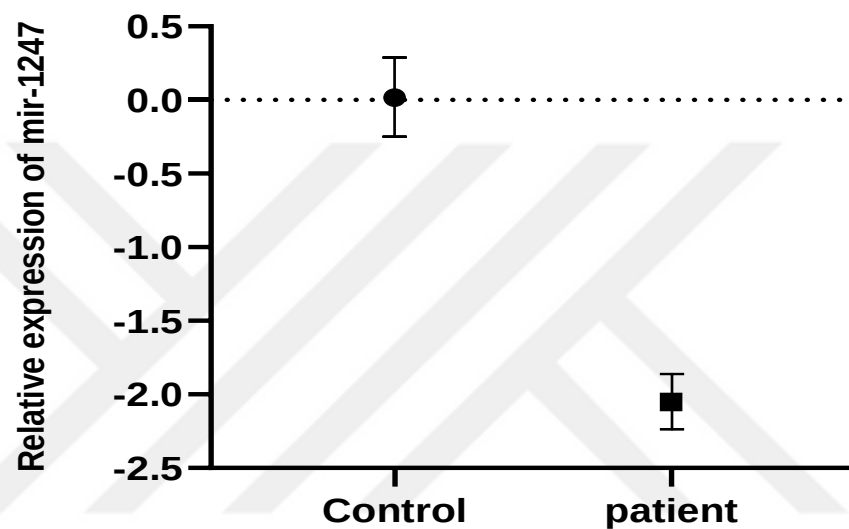


Figure 4-12: Bar and whisker chart representing the mean value of the expression level of miR-1247 in patients and control

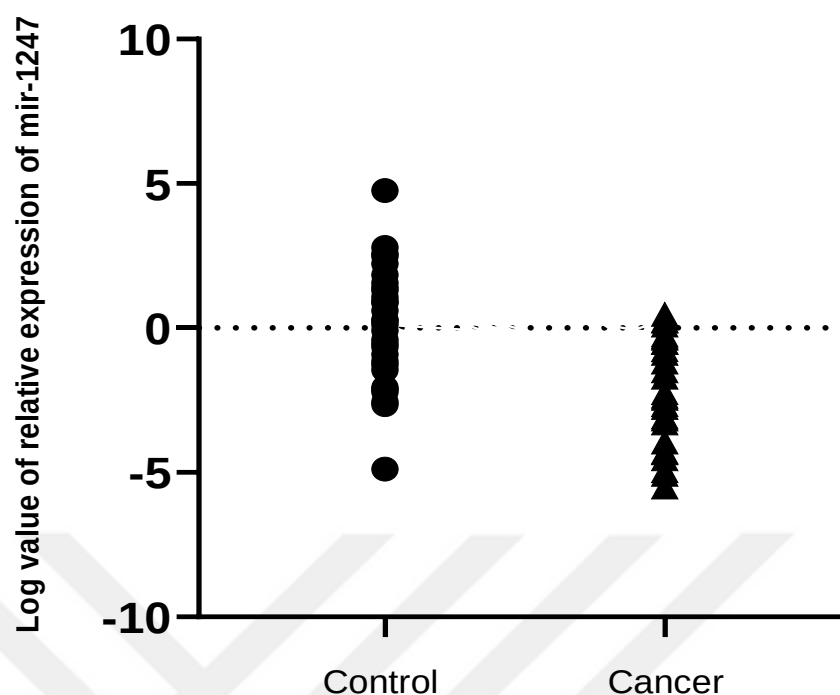


Figure 4-13: Logarithmic value of $2^{\Delta\Delta Ct}$ of the expression level of miR-1247 in cancer patients and control

4.2.6. MiR-92-a is upregulated in cancer patients

The obtained data from qRT-PCR, which operated to study the expression level of microRNA-92a within control, and CRC patients is included in raw data tables. The data is summarised as it contains the values of Ct, ΔCt , $\Delta\Delta Ct$, $2^{-\Delta\Delta Ct}$ and logarithmic value of subjects and the control subsets. Primarily, calculating the ratio of change in the expression level of miR-92a, revealing that there is a net 35 fold change between the control and patients group. The data indicates that the miR-92a expression level within the serum of CRC sufferers is 35 fold higher than those of control.

Bar and whisker chart (Figure 4-14) represents the mean value of the level of expression of mir-92a. We can notice that the affected level is below in the tumour patient and continue to decrease with the cancer progression. The significance value is identified with one-way ANOVA based on the control and patient's cancer stage, as indicated in (Table 4-29). The significance of the difference is indicated by a star; one mark if P- value (≤ 0.05), two-star if P-value (≤ 0.01), and three marks if P-value (≤ 0.001).

Figure (2-15) indicates the relative expression of miR-92a among the control group and colorectal cancer patients group. The significance value is represented with three-star above the bar, indicating that P-value is less than (0.001).

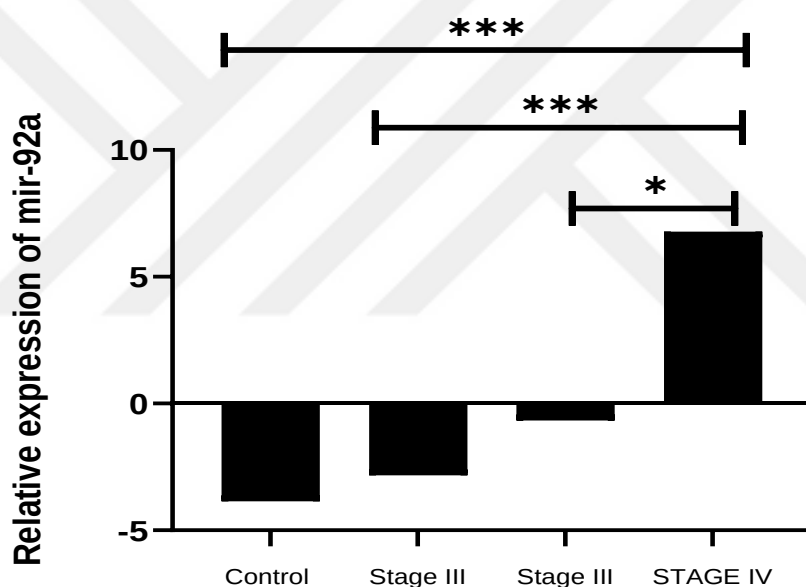


Figure 4-14: mean value of the expression level of miR-92a in control and CRC stages

Table 4-29: One-way ANOVA testing for significant difference for the expression level of miR-92a to the 0.05 level based on cancer

One-way ANOVA based on cancer stage of miR-92a Expression					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	609.246	3	203.082	7.883	0.000
Within Groups	1648.730	64	25.761		
Total	2257.976	67			

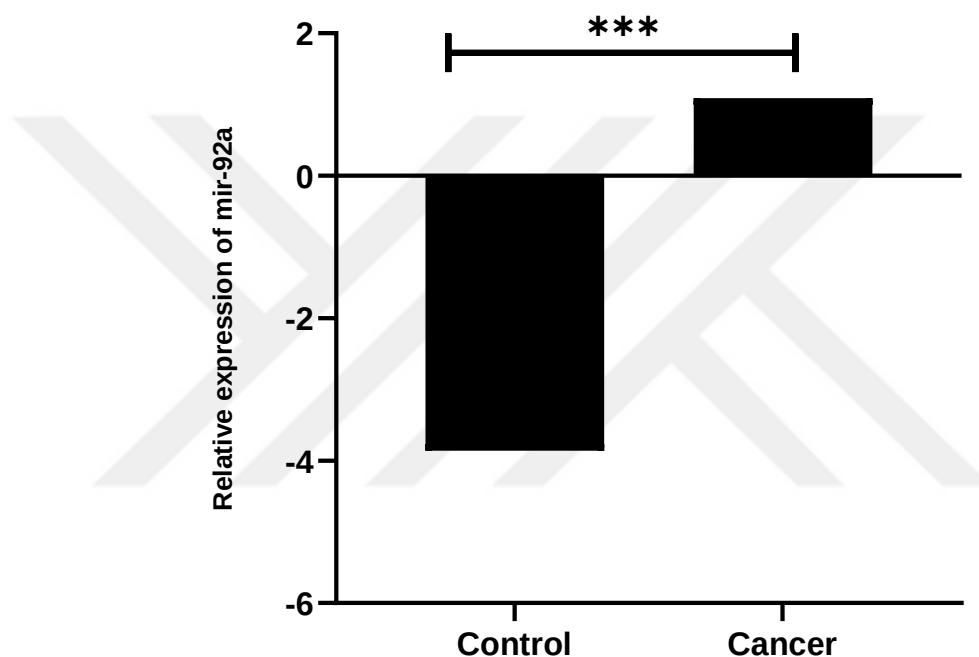


Figure 4-15: Relative expression of miR-92a, indicating the values and its significance

However, one-way ANOVA testing for notable variation for the expression level of miR-92a to the 0.05 level based on smoking, age, and gender reveals no significant level of differential expression with a value 0.97, 0.58 and 0.96, respectively; which consider a value more than 0.05 as it is insignificant.

Table 4-30: One-way ANOVA testing for significant difference for the expression level of miR-92a to the 0.05 level based on smoking

One-way ANOVA by smoking					
MiR-92a Expression					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.037	1	0.037	0.001	0.972
Within Groups	869.638	29	29.988		
Total	869.675	30			

Table 4-31: One-way ANOVA testing for significant difference for the expression level of miR-124 to the 0.05 level based on age

One-way ANOVA by age					
MiR-92a Expression					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	59.196	3	19.732	0.657	0.585
Within Groups	810.479	27	30.018		
Total	869.675	30			

Table 4-32: One-way ANOVA testing for significant difference for the expression level of miR- 92a to the 0.05 level based on gender

One-way ANOVA by gender					
MiR-92a Expression					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.063	1	0.063	0.002	0.964
Within Groups	869.612	29	29.987		
Total	869.675	30			

One-way ANOVA test significance of miR-92a to 0.05 in the control and CRC patient with different stages, shows no significant value as the value is all more than 0.05. Table (4-30) contains the result data of the test.

Table 4-33: One-way ANOVA test significance of miR-92a to 0.05 in the control and CRC patient with different stages

Multiple Comparisons						
Groups		Mean	Std. Error	Sig.	95% Confidence Interval	
		Difference (I-J)			Lower Bound	Upper Bound
Control	Stage II	-1.03	1.51	0.90	-5.01	2.95
	Stage III	-3.19	1.76	0.28	-7.83	1.46
	Stage IV	-10.63500000*	2.25	0.00	-16.56	-4.71
Stage II	Control	1.03	1.51	0.90	-2.95	5.01
	Stage III	-2.16	1.96	0.69	-7.34	3.02
	Stage IV	-9.60485063*	2.41	0.00	-15.96	-3.25
Stage III	Control	3.19	1.76	0.28	-1.46	7.83
	Stage II	2.16	1.96	0.69	-3.02	7.34
	Stage III	-7.44681818*	2.58	0.03	-14.24	-0.65
Stage IV	Control	10.63500000*	2.25	0.00	4.71	16.56
	Stage II	9.60485063*	2.41	0.00	3.25	15.96
	Stage III	7.44681818*	2.58	0.03	0.65	14.24

*, The mean difference is significant at the 0.05 level.

Plotting the logarithmic value of $2^{-\Delta\Delta Ct}$ of the expression level of miR-92a in cancer patients and control in (Figure 4-15), indicates the lower expression of miR-92a in cancer patients, comparing with the expression level among the control.

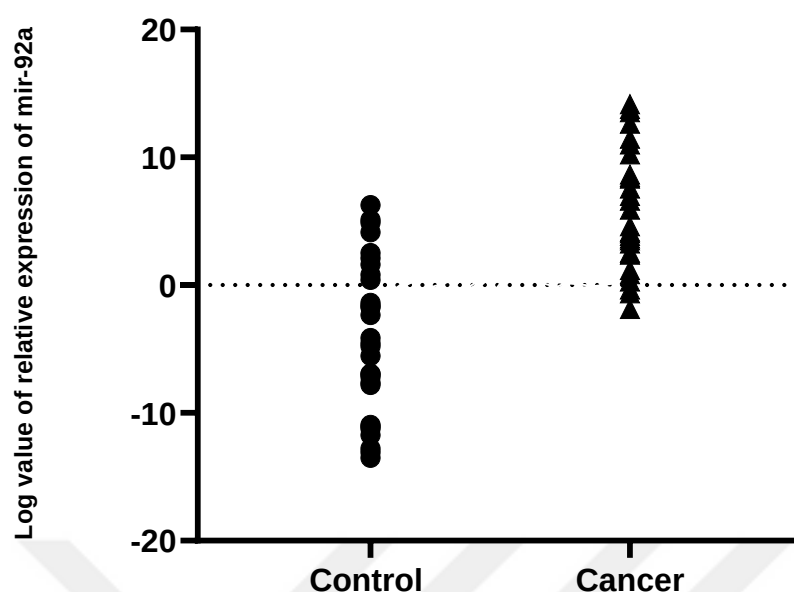


Figure 4-16: Logarithmic value of $2^{-\Delta\Delta Ct}$ of the expression level of miR-92a in cancer patients and control

As it is claimed that miR-92a is upregulated in cardiovascular diseases, the one-way ANOVA test was performed after classifying the patients into those who have cardiovascular disease and colorectal cancer patients with no cardiovascular disease. The result indicates no significance value.

Table 4-34: One-way ANOVA testing for significant difference for the expression level of miR-92a to the 0.05 level based on health status

One-way ANOVA based on health status					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.974	1	11.974	0.405	0.530
Within Groups	857.701	29	29.576		
Total	869.675	30			

In conclusion, the fold change of all selected miRNAs, the figure (4-16) represents the fold change of six micro-RNAs; miR-195, miR-224, miR-214, miR-1247, miR-126,

and microRNA -92a. We can see that mir-195 is the most decreased miRNA among the studied ones with a fold change (- 18). Besides, it is noticeable that four out of the six miRNA are down-regulated, while two of them are upregulated in cancer patients with 35 and 6.6 fold change for miR-92a and miR-214, respectively.

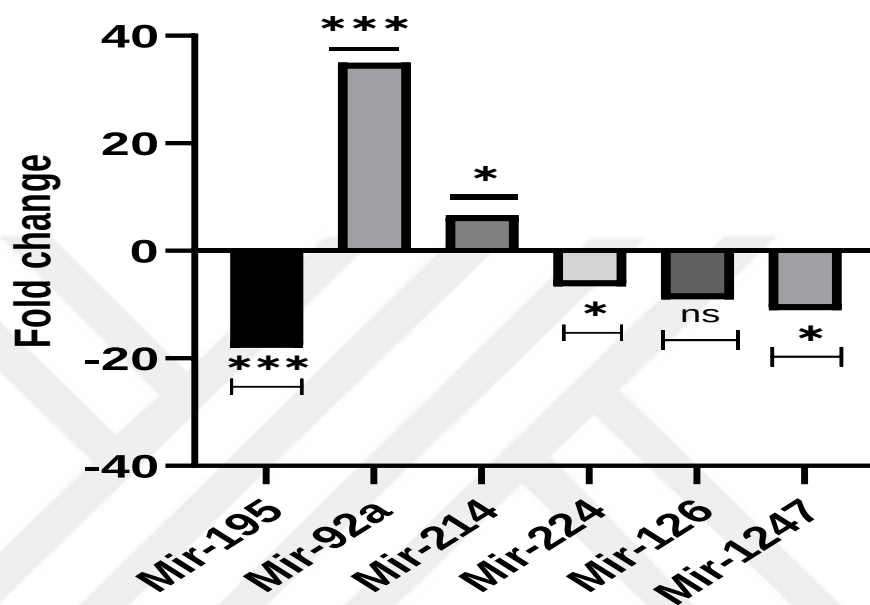


Figure 4-17: fold change of the six studied miRNA in cancer patients compared with the control

5. DISCUSSION

The early diagnosis of colorectal cancer allows a better treatment approach and a better outcome. Our study is one of the several studies conducted and ongoing on the performance of microRNA in tumour development and forecast. The hope of the ability to use a reliable non-invasive approach for diagnosis is very promising. The evidence from the previous research, which was also confirmed by the following ones is encouraging other researchers to conduct research more in-depth about this subject. This study is the first in assessing those microRNA among the Turkish population to assess the possibility of using them for diagnostic purpose.

5.1. Serum miR-195 as a diagnostic biomarker of CRC

Concerning our investigation, it observed that the serum level of microRNA-195 expression is reduced in the cancer patients contrasted with the control group. With an overall fold change reach up to -18 times more depressed in carcinoma subjects than the control. Moreover, the level is even lowering with the stage progression. Referring to the table (4-3), we can notice that the mean of stage II cancer is -2.49 while among stage 4 patients is -8.49, that means about fourfold lower with the metastatic stage.

This study is confirming the result of several previous studies which claimed that miR-195 has an anti-cancer role as it is functioning as a neoplasm inhibitor gene. Its reduced level is found to be linked to the grade of malignancy. The lower the miR-195 level is the further invasive cancer develop. Su et al. (2016), presents that in human cancer, an area on chromosome "17p13.1" in which the miR-195 is discovered, it is usually deleted. Thus, almost all carcinoma has a lower state of representation of miR-195. Wang, F. et al. (2015) ascertained that miR-195 downregulation in thyroid carcinoma. Similarly, depressed expression state of miR-195 was distinguished in tongue cancer tongue (Jia et al. 2013), oesophagus (Fu et al. 2013), gastric Cancer (Deng et al. 2013), hepatocellular carcinoma (Wang, M. et al. 2015), glioblastoma (Susluer et al. 2015), prostate cancer (Cai et al., 2015). Also, in osteosarcoma, bladder, besides in CR Carcinoma (Han et al.; Itesako et al.; Yang et al., 2015). Our result is also consistent with

Wang.X et al. (2012), who exhibited a notable drop in miR-195 level in patients with metastasises and an advanced tumour stage.

The concentration of serum expression of microRNA 195 in CRC sufferers was prominent and reliable and correlated with advanced stages and metastasis. We have to assess its specificity to consider it as a specific biomarker for CRC. We have to increase the samples number and to assess the patients after the treatment period to compare the result before and after the treatment.

On the other hand, we did not find a significant value among the smoker and non-smoker patients with the level of miR-195 expression. Also, there implied no impact of age and gender on the serum level of the patient. The reason could be because the stages numbers are not consistent as the overall number was as the following; 6 specimens from cases with "stage IV"; 16 individuals with "stage III" and 19 blood specimens from patients with grade II. Though, some of these samples were not reliable and excluded either because of the storage situation or because of approach error of the protocol.

5.2. Serum miR-224 downregulation in CRC

MiR-224 was proposed to be downregulated in numerous neoplastic growth, that is also confirmed by our study, which represents a lessening in the expression level of miR-224 among CRC patients. Furthermore, others represent a contrary result, conflicting with our finding.

As the miR-224 is acted via the cooperation with apoptosis inhibitor-5 transcript expression to control the cancerogenesis (Wang et al., 2008), multiple other studies published that miR-224 increased in many other carcinoma varieties and even it id found to be connected with the progression of the condition. Wang et al. (2019) confirmed that miR-224 performs a crucial part in cellular functions in adjusting the target PTEN and downstream signal PI3K. Zheng et al. (2019) noticed that miR-224 is extremely overexpressed in TNBC carcinoma cells. Similarly, the creation of miRNA-224 was raised in blood from HCC cases matched to subjects with liver cirrhosis and healthy individuals, correlated by the tumour extension (Shehab-Eldeen et al., 2019). Moreover, Zheng et al., (2019) by examination of the data from the Cancer Genome Atlas noted that

in glioblastoma, there is a correlation between the of microRNA-224 downregulation and poor outcome.

Regarding the CRC, Several studies proposed increase of miR-224 in CRC were reviewed by Ding et al. (2018); they indicate that miR-224 is induced in colorectal cancer and further it is related with the advance grade and aggression. Brunet Vega et al. (2013) propose that using reverse transcription and qRT-PCR to analyse 11 miRNAs, including miR-224, was confirmed as significantly overexpressed in tumour samples when compared with standard samples even though it is increased in the cancer tissue samples but was not consistently measurable in serum.

With our study, the down-regulation of miR-224 was apparent with an overall fold change -6.4, and the significance value was lower than 0.05, which indicate the meaningful distinction among the diseased subjects and control. A similar issue was proposed by Ke et al. (2014), miR-224 represses CRC migration through Cdc24 affecting; the expression level of miR-224 was more depressed in CRC tissue sample in comparison with healthy tissues. Additionally, we detected that the level of microRNA 224 expression is related amidst the cancer stage but is not connected to age, gender, smoking and the clinical state of the patients.

Interestingly, among our samples there are about four patients have a significantly higher level of miR-224 which was about 100 times above the other patients in the group, that could be subjected to a case study research to compare them and identify if the targeted pathway among those patients were different.

It could be concluded that the dual action of miR-224 requires more study because the result was conflicting depends on which pathway is activated by miR-224; conduction more study combining the expression level study with the target pathway would increase our understanding of this miR-224.

5.3. Serum miR-214 as a diagnostic biomarker of CRC.

MicroRNA 214 is identified to accentuate several neoplasm-related signalling pathways in various malignancies. miR-214 reportedly executed a task in numerous

carcinoma prototypes and was involved in multiple cascades (Sharma and colleagues, 2015; Penne et al., 2015). As reviewed through Cagle et al. (2019), the function of miR-214 is specific to the type of the cells, including its distinct roles relying on its distinct aimed genes; miR-214 might be either tumour suppressor or oncogenic functions in different tumour samples, such as melanoma, ovarian, and breast cancer.

Our result shows that miR-214 is elevated in cancer subjects with a fold change 6.6 comparing with control. This finding was consistent with the result claimed by Zhao et al. (2018), that miR-214 promotes stomach carcinoma propagation, movement and aggression. Moreover, It has been revealed that the upregulation of microRNA 214 in osteosarcoma contributes to radio-resistance (Li y et al., 2019). The reduced state of miR-214 significantly repressed the breast cancer cells survival, movement and aggression, and inhibited the PI3K/Akt/mTOR pathway (Zhang Y, 2019). It was noticed that miR-214 is up-regulated within osteosarcoma lines and specimen (Zhao x et al., 2019), and CRC victims (reviewed in Ding et al., 2019).

On the contrast, our study finding is contrary to other studies which proposed the suppressing task of microRNA 214 as they stated that microRNA 214 is downregulation in carcinoma of the cervix as it is inhibiting cell propagation, immigration, intrusion and enhances drug responsiveness. Similarly, miR-214 through regulating Caspase 1-mediated pyroptosis inhibits cell generation and movement of gliomas and brain tissue. Moreover, It has explicated that it operates as a tumour suppressor in colon malignancy (Yang et al. 2008), stomach tumour (Wang R et al., 2019), and OSCC (Wang X et al., 2019). Researchers indicate that expanding microRNA 214 levels in the metastatic site of the bone might inhibit prostate cancer infiltration (Duan et al., 2019),. The overexpression of miR 214 increases E-cadherin plus tissue inhibitor of metalloproteinases-2 expressions and decreases MMP2 also MMP 9 expression that inhibiting cell intrusion and immigration, (Shan et al., 2019).

We noticed that miR-214 level is raised in the serum of cases, and there is a relation with the grade of the disorder; the most eminent level was among stage IV cancer patients. However, gender, smoking, age does not affect the pattern of elevation.

Despite there is a conflicting result, some other studies indicate the dual action of miR-214 according to the activated pathway. The result is promising as a prognostic factor if two reading compared before and after treatment, it could notice a change in the serum expression level, indicating an improvement.

5.4. MiR-126 is downregulated in the serum of cancer patients.

MiR-126 has been distinguished as a critical cellular factor in the evolution of several cancers (Zhang., 2013). reduction of MicroRNA 126 within malignant vs nonmalignant tissues was published for lung cancer. (Cho et al., 2009), in colon cancer (Guo et al., 2008). Some results showed a low representation of both microRNA-126-3p and -5p in adenocarcinoma of Lung and even lower in the later stage of cancer and metastasis, miRNA-126 has a crucial task in LUAD growth (Chen et al., 2019).

The status of miR-126 expression was remarkably further depressed in subjects with stage three and four malignant gliomas than in cases with the first and second stage, using miR-126 inhibitor significantly increased the invasiveness of the malignant cell, compared with control, in this study, they concluded that miR-126 by inhibiting MTCP1 reduces the immigration of malignant glioma (Han et al., 2018).

Alteration within the expression levels of this miRNA has been announced in several tumour cells such as those arising from the breast (Nargniri at el., 2008), lung (Crawford., 2018), cervix (Wang X at el 2009), liver (Ladeiro at el., 2008), prostate (Musieinko at el., 2010), colon (Diaz at el., 2008), oesophagal (Hu at el., 2011), and leukaemia (Li at el., 2008).

Our result is confirming the downregulation of miR-126 among carcinoma patients. We determined that miR-126 is nine times lower in the samples of cancer cases corresponded with the healthy. Although it was a clear distinction in the value among the cancer cases and the control, the significance value indicates an insignificant difference. Also, the level of expression of miR-126-3p was not affected by age, gender, smoking, or the health situation of the patients. There was no notable value in the correlation of the expression level with different cancer stages.

We can conclude our result like that, miR-126 is depressed in the cancer subjects in comparison with the control group; the result was compatible with the prior investigations; the opportunity of using microRNA 126-3p as a bioindicator of the diagnosis of CRC among Turkish population is proposed.

5.5. MiR-92a expression level is elevated in the serum of cancer patients.

The upregulation of miR-92a was significantly reported in several malignancies; Volinia et al., (2006) identified expression of microRNA in about 540 neoplasm specimens for instance colon, mammary gland, stomach, lung, prostate, and pancreas malignancies, also recognised the elevation of miR-92a in cancerous tissues. Within the members of MicroRNA 17–92 family that found to be increased in the serum of gastric carcinoma as well as in intestinal metaplasia (Mihailovich et al., 2015). Some analysis explained that miR-92a effected endothelial inflammation (Liu et al., 2016), and controlling levels of cholesterol and Golgi volume through protein secretion (Loh et al., 2017).

Our finding is similar to the previously reported results in term of expression level in colon cancer tissue, but we further detected the overexpression in the serum of Turkish patients comparing with the control. Similarly to Si et al., (2013) who identified the circulating level of miR-92a who was proposed to be used as a novel biomarker. It has been found that miR-92a is an oncogene promoting the propagation, movement and aggression of oesophageal squamous cell tumour by regulating PTEN.

The transcription level in the serum of colorectal carcinoma cases is 35 folds higher than these of the control subset. However, there was no notable alteration in relation to the gender difference, Age and smoking. On the other hand, the serum level is increasing with advancing stage of the disease, which indicates its function in the poor prognosis and the migration of the Colorectal Cancer. Yamada et al. (2013), explicated that the circulating plasma concentration of microRNA 92a is markedly enhanced in mice displaying xenografts of colon carcinoma. However, our result is conflicting that the expression concentration of microRNA 92a is displayed at depressed level in the malignancy of ovary (Ohayagi-Hara et al., 2013).

Interestingly, the higher serum miR-92a state was found to be linked with elevating the risk of acute coronary syndrome in coronary heart disease cases with type-2 DM (Wang, W., 2019). This assumption arises a doubt concerning the specificity of miR-92a among our study group; as about half of the patients have either DM or CAD or both, which could have affected the result. Therefore, we perform one-way ANOVA to differentiate between the expression level in the patient complaining of DM and cardiovascular, and those who have no such a disease.

5.6. MicroRNA-1247 expression level is down-regulated among cancer patients.

MicroRNA-1247 (miR-1247) is one of the miRNAs that has gotten particular concentration in tumour investigation as its expression is altered in several malignancies, for instance, pancreatic cancer, NSCLC, prostate cancer, hepatocellular cancer (HCC), childhood neuroblastoma, and osteosarcoma. However, there is no study on this miR-1247 in colorectal cancer patient to detect the serum level of this particular miRNA among cancer patients. Our research indicates that serum expression level of miR-1247 is remarkably diminished among tumour patients contrasted with healthy control individual.

MiR-1247 expression level is 2.05 times lower level in the serum of CRC cases, in contrast with the control group. Moreover, it was significantly correlated with cancer stages; the lowest value was among stage IV colorectal carcinoma patients, while among those of stage II have a considerably higher value.

Similarly, other studies showed that microRNA-1247 is downregulated in the pancreatic cancer tissue as compared to matching benign tissues, as well as in the tissues and cells associated with NSCLC. MiR-1247 is also decreased in squamous carcinoma cancer tissue and lung adenocarcinoma than the healthy tissues (Zheng et al., 2016).

Researchers also disclosed the significant downregulation of microRNA 1247-5p in the cells and tissues associated with Hepatocellular cancer (Chu et al., 2017).

Studies have shown microRNAs have a critical function in the underlying pathogenesis neuroblastoma. Wu et al. (2018) announced that miR-1247 is

downregulated in the tissues and cells related to neuroblastoma. They additionally stated the presentation of microRNA 1247 could profoundly suppress the propagation of cells, and induce the arrest of the cell cycle at G0/G1 point along with cell programmed cell death of neuroblastoma. The expression of miR-1247 is considerably reduced in bladder malignancy (Zhu et al., 2018).

MicroRNA 1247 was found to have a role in prostate cancer. In advanced stages, it becomes CRPC, which is incurable. MicroRNA 1247–5p interacts with MYCBP2's 3'UTR and results in its reduction in prostate cancer. It can also slightly reduce SOX9 mRNA in cell-lines of prostate cancer (Scaravilli et al., 2015).

On the contrast, the conflicting result was announced by other studies, indicating an increased level of expression of microRNA 1247 is a predicting factor in increasing the overall survival and reoccurrence-free survival in case of pancreas malignancy as it is negatively linked to the staging of the tumour (Shi et al., 2014).

Despite the insignificant correlation of age, gender or smoking with the serum expression level of microRNA1247; microRNA 1247 has -11 fold change might be used as a novel biological marker for the detection of colorectal neoplasm. As a conclusion to our findings, microRNA126-3p, microRNA195, microRNA 224 and microRNA 1247 were downregulated in the serum of CRC patients correspond to those of control with a fold change (- 9, - 18, -6.6, and - 11, respectively). With the most reduced micro-RNA among the six studied miRNA is miR-1247. However, miR-214 and miR-92a were upregulated with a fold change (6.6 and 35, respectively).

There was a limitation to our studies such as the number of samples and the inconsistency of grouping; as there was a different number of each stage with no stage 0 & I colorectal cancer, six patients with stage IV, 17 with stage three and 19 patients have grade two colorectal cancer. There difference in the number of cases could lead to inconsistency in the result in term of comparing the expression level among the stages of colorectal cancer.

Repeating the experiments with a higher number of patients and repeating the analysis after treatment to identify the variation in the expression level of those microRNA. Studying the targeting pathway would be efficient as well to recognise the

pathway that causes the miRNA to be down-regulated or up-regulated, in particular in micro-RNA with conflicting results among the conducted studies.

Proving the availability of using microRNA as an invasive diagnostic and prognostic approach, would help in early diagnosis and a better outcome, which would reduce the cost of treatment. Moreover, it would be such a relief to the patients who are suffering from the invasive diagnostic method.



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RAW DATA

The database concerning of the colorectal cancer patients

Patient No.	sex	Age	Stage TNM	Smoking	Other diseases	FH
1	M	64	2	+	Chronic obstructive pulmonary disease (COPD)	-
2	M	65	4	-	COPD	-
3	M	52	3	-	Anaemia	-
4	M	67	2	-	-	-
5	M	33	3	-	-	+
6	M	30	2	-	-	+
7	M	67	4	+	-	+
8	F	85	2	-	DM	-
9	F	62	4	-	DM, HT	+
10	M	73	4	+	-	-
11	M	58	3	-	DM, COPD	+
12	M	61	4	+	DM	-
13	M	84	3	-	HT	-
14	M	68	2	+	Aortic insufficiency	-
15	M	73	3	+	DM	-
16	M	69	3	-	COPD, HT	-
17	M	70	4	+	DM	+
18	M	54	2	-	-	-
19	F	49	3	-	-	-
20	F	70	2	-	COPD	-
21	M	69	2	-	HT	-
22	M	58	3	-	DM	+
23	M	80	2	+	COPD	-
24	F	69	2	-	DM	-
25	F	56	2	+	-	-
26	F	48	2	-	-	-
27	M	51	3	+	DM, HF, CKD	-
28	M	55	2	+	Coronary artery disease, HF	-
29	M	61	3	-	-	+
30	F	48	3	+	-	-
31	M	79	2	-	PARKINSON	-
32	F	87	2	-	Coronary artery disease	-
33	M	67	2	+	-	-
34	F	73	3	-	DM	-
35	M	54	2	+	-	-
36	M	73	3	+	DM	-
37	M	75	2	-	DM, CAD	-
38	M	71	2	-	Coronary artery disease	-
39	M	24	3	+	-	-
40	F	58	3	-	Rheumatoid Arthritis	-

The data obtained from qRT-PCR, along with the primary calculation obtained by using the equations mentioned above 3-1, 3-2 and 3-3.

The primary calculation of MiR-195 expression level										
Patients						Control				
Patient No	Ct	Δ ct	$\Delta\Delta$ ct	$2^{\Delta\Delta\text{ct}}$	Log value	Ct	Δ ct	$\Delta\Delta$ ct	$2^{\Delta\Delta\text{ct}}$	Log value
1	21.06	3.42	-0.69	1.6	0.69	23.46	3.46	-0.65	1.56	0.65
2	26.02	9.14	4.76	0.0	-4.76	30.38	4.07	-0.31	1.24	0.31
3	33.84	16.72	12.35	0.0	-12.34	28.71	-3.14	-7.52	182.95	7.52
4	29.68	7.90	3.52	0.1	-3.52	28.37	2.50	-1.87	3.66	1.87
5	26.22	4.32	-0.05	1.0	0.05	24.11	0.92	-3.45	10.93	3.45
6	26.56	10.38	6.01	0.0	-6.00	25.37	-1.17	-5.55	46.73	5.55
7	31.00	4.99	0.62	0.7	-0.62	29.98	-2.92	-7.30	157.41	7.30
8	36.05	13.86	9.49	0.0	-9.49	29.34	2.41	-1.96	3.89	1.96
9	29.56	11.30	6.92	0.0	-6.92	29.27	-4.10	-8.47	354.89	8.47
10	30.45	9.28	4.91	0.0	-4.90	31.34	8.46	4.08	0.06	-4.08
11	26.17	6.81	2.44	0.2	-2.44	28.11	10.59	6.21	0.01	-6.21
12	28.87	12.43	8.06	0.0	-8.06	29.20	7.85	3.48	0.09	-3.48
13	24.14	6.88	2.50	0.2	-2.50	26.63	-1.64	-6.01	64.59	6.01
14	25.94	8.03	3.65	0.1	-3.65	21.44	3.48	-0.90	1.87	0.90
15	31.28	16.20	11.82	0.0	-11.82	30.19	-3.06	-7.43	172.45	7.43
16	29.42	10.70	6.32	0.0	-6.32	19.28	0.72	-3.66	12.63	3.66
17	25.77	6.20	1.82	0.3	-1.82	30.22	8.36	3.98	0.06	-3.98
18	25.04	8.94	4.57	0.0	-4.56	27.07	9.34	4.97	0.03	-4.97
19	28.39	8.60	4.23	0.1	-4.23	25.13	6.75	2.38	0.19	-2.38
20	31.18	12.40	8.03	0.0	-8.02	30.84	-1.96	-6.34	80.92	6.34
21	28.43	6.19	1.82	0.3	-1.82	29.32	6.89	2.52	0.17	-2.52
22	25.22	8.74	4.37	0.0	-4.36	29.36	-1.59	-5.96	62.24	5.96
23	30.65	9.68	5.30	0.0	-5.30	30.90	-0.84	-5.21	37.01	5.21
24	25.44	9.00	4.63	0.0	-4.62	27.37	8.48	4.11	0.06	-4.11
25	22.43	4.12	-0.26	1.2	0.26	28.47	9.94	5.57	0.02	-5.57
26	31.00	13.36	8.99	0.0	-8.99	30.91	2.53	-1.84	3.59	1.84
27	22.58	7.05	2.67	0.2	-2.67	31.29	-0.06	-4.43	21.56	4.43
28	30.11	13.94	9.56	0.0	-9.56	31.07	9.93	5.56	0.02	-5.56
29	32.29	8.28	3.91	0.1	-3.90	30.58	5.75	1.37	0.39	-1.37
30	25.43	8.47	4.09	0.1	-4.27	24.12	8.04	3.66	0.08	-3.66
31	25.43	8.65	4.27	0.1	-2.98	28.49	4.52	0.15	0.90	-0.15
32	26.49	7.36	2.99	0.0	-6.71	33.01	9.17	4.80	0.04	-4.80
33	27.55	11.09	6.71	0.0	-4.60	28.73	5.32	0.95	0.52	-0.95
34	27.35	8.98	4.61	9.3	3.21	31.81	7.84	3.46	0.09	-3.46
35	24.08	1.16	-3.22	0.0	-10.31	29.77	9.98	5.61	0.02	-5.61

The data which is obtained from qRT-PCR of miR-214, along with the primary calculation using the equations mentioned above

The primary calculation of miR-214 expression level										
	Patients					Control				
	Ct	Δ Ct	$\Delta\Delta$ Ct	$2^{\Delta-\Delta\Delta$ Ct}	Log value	Ct	Δ Ct	$\Delta\Delta$ Ct	$2^{\Delta-\Delta\Delta$ Ct}	Log value
1	24.82	7.18	5.22	5.45	2.45	26.53	6.53	4.57	0.07	-3.84
2	21.02	4.13	2.24	5.51	2.46	26.83	0.52	-1.38	0.26	-1.96
3	20.45	3.34	1.44	5.59	2.48	22.05	-9.80	-11.69	0.30	-1.74
4	26.74	4.96	3.07	5.70	2.51	25.25	1.39	-0.51	0.62	-0.70
5	25.51	3.62	1.73	5.77	2.53	22.19	-1.00	-2.89	0.65	-0.63
6	27.55	11.37	9.48	5.88	2.56	23.68	-2.86	-4.76	0.74	-0.43
7	27.60	1.59	-0.30	5.78	2.53	23.74	-9.16	-11.05	0.90	-0.15
8	26.09	3.91	2.02	5.96	2.58	26.24	-0.69	-2.58	1.25	0.32
9	25.32	7.05	5.16	6.08	2.60	27.22	-6.15	-8.05	1.37	0.45
10	26.77	5.61	3.71	6.11	2.61	26.83	3.95	2.05	1.66	0.73
11	22.82	3.46	1.57	6.18	2.63	25.54	8.02	6.12	1.65	0.72
12	26.34	9.91	8.01	6.34	2.66	25.42	6.07	4.18	1.50	0.59
13	28.08	10.82	8.92	6.28	2.65	31.26	3.00	1.10	1.41	0.49
14	26.74	8.82	6.93	6.19	2.63	28.21	10.25	8.35	1.42	0.51
15	27.95	12.87	10.97	6.16	2.62	24.57	-8.68	-10.57	1.16	0.22
16	26.27	7.55	5.65	5.97	2.58	27.70	9.14	7.24	1.61	0.69
17	25.62	6.05	4.15	5.99	2.58	24.00	2.14	0.24	1.39	0.47
18	27.87	11.76	9.87	6.06	2.60	26.39	8.66	6.76	1.44	0.52
19	27.94	8.16	6.26	5.90	2.56	28.13	9.75	7.86	1.21	0.27
20	25.94	7.16	5.27	5.88	2.56	24.67	-8.13	-10.03	0.90	-0.15
21	27.13	-4.00	-5.89	5.91	2.56	25.34	5.48	3.58	1.42	0.51
22	26.99	4.76	2.86	6.50	2.70	25.99	3.56	1.67	1.32	0.40
23	20.55	4.07	2.18	6.69	2.74	25.07	-5.87	-7.77	1.30	0.38
24	28.77	7.79	5.90	6.94	2.80	26.11	-5.63	-7.53	1.80	0.85
25	27.26	10.83	8.94	7.00	2.81	28.48	9.60	7.70	2.35	1.23
26	28.65	10.34	8.45	6.88	2.78	25.72	7.19	5.30	2.02	1.01
27	25.32	7.69	5.79	6.78	2.76	27.80	-0.58	-2.48	1.80	0.85
28	28.03	12.50	10.60	6.85	2.78	24.41	-6.94	-8.84	2.10	1.07
29	24.55	8.39	6.49	6.56	2.71	27.60	6.47	4.57	2.94	1.56
30	28.14	4.13	2.24	6.57	2.72	24.48	-0.35	-2.25	2.81	1.49
31	29.01	12.05	10.16	6.96	2.80	29.02	8.51	6.62	3.27	1.71
32	27.56	10.78	8.89	6.64	2.73	27.93	8.34	6.44	2.93	1.55
33	28.43	9.31	7.41	6.39	2.68	25.63	6.32	4.42	2.54	1.35
34	26.71	10.25	8.35	6.26	2.65	27.03	-0.66	-2.55	2.31	1.21
35	26.53	8.16	6.27	5.96	2.58	26.81	5.92	4.03	3.00	1.59
36	26.63	3.71	1.81	5.91	2.56	25.18	3.35	1.46	2.83	1.50
37	28.72	12.41	10.52	6.74	2.75	28.13	9.48	7.59	3.10	1.63
38	26.15	7.85	5.96	5.79	2.53	27.49	3.95	2.06	1.98	0.99
39	28.30	11.99	10.10	5.73	2.52	24.59	5.93	4.04	1.96	0.97
40	28.08	9.78	7.89	3.55	1.83	26.84	3.30	1.41	0.92	-0.13

The data obtained from qRT-PCR and its calculation of the expression pattern of miR-224 among cancer and control group

The primary calculation of miR-224 expression level									
Patients					Control				
Ct	Δ ct	$\Delta\Delta$ ct	$2^{-\Delta\Delta$ ct	Log value	Ct	Δ ct	$\Delta\Delta$ ct	$2^{-\Delta\Delta$ ct	Log value
31.00	4.99	4.99	0.29	-1.81	29.98	-2.92	-2.92	69.05	6.11
36.05	13.86	13.86	0.00	-10.67	29.34	2.41	2.41	1.73	0.79
29.56	11.30	11.30	0.00	-8.09	29.27	-4.10	-4.10	157.22	7.30
30.45	9.28	9.28	0.01	-6.08	27.34	4.46	4.46	0.42	-1.26
26.17	6.81	6.81	0.08	-3.61	28.11	10.59	10.59	0.01	-7.39
28.87	12.43	12.43	0.00	-9.23	29.20	9.85	9.85	0.01	-6.65
24.14	6.88	6.88	0.08	-3.67	26.63	-1.64	-1.64	28.61	4.84
25.94	8.03	8.03	0.04	-4.82	25.44	7.48	7.48	0.05	-4.28
31.28	16.20	16.20	0.00	-12.99	29.19	-4.06	-4.06	152.79	7.26
29.42	10.70	10.70	0.01	-7.49	30.28	11.72	11.72	0.00	-8.52
37.56	6.43	6.43	0.11	-3.23	28.12	8.26	8.26	0.03	-5.06
23.00	0.77	0.77	5.38	2.42	20.13	-2.29	-2.29	45.09	5.49
36.66	20.18	20.18	0.00	-16.97	34.80	3.86	3.86	0.63	-0.66
35.19	18.76	18.76	0.00	-15.56	28.19	9.31	9.31	0.01	-6.11
23.05	4.74	4.74	0.34	-1.54	18.00	-0.53	-0.53	13.29	3.73
26.33	8.70	8.70	0.02	-5.50	26.57	-1.80	-1.80	32.09	5.00
32.14	15.98	15.98	0.00	-12.78	31.91	10.78	10.78	0.01	-7.58
29.53	11.22	11.22	0.00	-8.02	25.67	7.14	7.14	0.07	-3.94
25.43	7.80	7.80	0.04	-4.59	25.93	-2.45	-2.45	50.15	5.65
25.41	9.87	9.87	0.01	-6.67	25.67	-5.68	-5.68	470.31	8.88
24.70	8.53	8.53	0.02	-5.33	20.55	-0.59	-0.59	13.81	3.79
29.56	5.55	5.55	0.20	-2.35	28.47	3.63	3.63	0.74	-0.43
26.51	9.55	9.55	0.01	-6.34	27.26	6.76	6.76	0.09	-3.56
28.13	11.35	11.35	0.00	-8.14	25.26	5.66	5.66	0.18	-2.46
29.40	10.27	10.27	0.01	-7.07	28.77	9.45	9.45	0.01	-6.25
27.75	11.29	11.29	0.00	-8.08	27.22	-0.47	-0.47	12.72	3.67
24.18	5.81	5.81	0.16	-2.60	23.30	2.41	2.41	1.73	0.79
29.18	6.25	6.25	0.12	-3.05	29.77	7.94	7.94	0.04	-4.74
26.92	10.62	10.62	0.01	-7.41	22.94	4.29	4.29	0.47	-1.09
29.08	10.78	10.78	0.01	-7.58	23.71	0.17	0.17	8.17	3.03
26.16	7.86	7.86	0.04	-4.66	22.67	-0.87	-0.87	16.80	4.07
31.00	4.99	4.99	0.29	-1.80	29.98	-2.92	-2.92	69.05	6.11
36.05	13.86	13.86	0.00	-10.66	29.34	2.41	2.41	1.73	0.79
29.56	11.30	11.30	0.00	-8.09	29.27	-4.10	-4.10	157.22	7.30
30.45	9.28	9.28	0.01	-6.08	27.34	4.46	4.46	0.42	-1.26
26.17	6.81	6.81	0.08	-3.61	28.11	10.59	10.59	0.01	-7.39
28.87	12.43	12.43	0.00	-9.23	29.20	9.85	9.85	0.01	-6.65
24.14	6.88	6.88	0.08	-3.67	26.63	-1.64	-1.64	28.61	4.84
25.94	8.03	8.03	0.04	-4.82	25.44	7.48	7.48	0.05	-4.28
31.28	16.20	16.20	0.00	-12.99	29.19	-4.06	-4.06	152.79	7.26
29.42	10.70	10.70	0.01	-7.49	30.28	11.72	11.72	0.00	-8.52

The data obtained from qRT-PCR and its calculation of the expression pattern of miR-126 among cancer and control group

qRT-PCR data of miR-126 expression									
Patients					Control				
Ct	Δ ct	$\Delta\Delta$ ct	$2^{-\Delta\Delta$ ct	Log value	Ct	Δ ct	$\Delta\Delta$ ct	$2^{-\Delta\Delta$ ct	Log value
25.63	3.94	0.88	0.5	-0.27	25.63	5.64	2.58	0.2	-2.6
29.44	7.67	4.61	0.0	-1.39	29.44	-4.16	-7.22	148.7	7.2
27.33	11.71	8.65	0.0	-2.60	27.33	1.02	-2.04	4.1	2.0
26.88	6.98	3.92	0.1	-1.18	26.88	-4.97	-8.03	261.4	8.0
29.08	-3.08	-6.14	70.6	1.85	29.08	5.21	2.15	0.2	-2.1
21.62	3.28	0.22	0.9	-0.07	21.62	-1.57	-4.63	24.7	4.6
28.73	11.92	8.86	0.0	-2.67	28.73	2.19	-0.87	1.8	0.9
26.96	-1.91	-4.97	31.3	1.50	26.96	-5.94	-9.00	513.1	9.0
34.06	7.03	3.97	0.1	-1.19	34.06	7.14	4.07	0.1	-4.1
29.5	7.54	4.48	0.0	-1.35	29.5	-3.87	-6.93	122.1	6.9
25.46	2.35	-0.71	1.6	0.21	25.46	2.58	-0.48	1.4	0.5
28.1	8.54	5.48	0.0	-1.65	28.1	10.58	7.52	0.0	-7.5
28.55	3.01	-0.05	1.0	0.01	28.55	9.20	6.14	0.0	-6.1
28.12	10.11	7.05	0.0	-2.12	28.12	-0.15	-3.21	9.2	3.2
21.26	4.20	1.14	0.5	-0.34	21.26	3.29	0.23	0.9	-0.2
27.26	12.35	9.29	0.0	-2.80	27.26	-5.99	-9.05	529.0	9.0
26.85	9.61	6.55	0.0	-1.97	26.85	8.29	5.23	0.0	-5.2
29.24	1.48	-1.58	3.0	0.48	29.24	7.38	4.32	0.1	-4.3
22.32	4.77	1.71	0.3	-0.51	22.32	4.59	1.53	0.3	-1.5
26.12	7.44	4.38	0.0	-1.32	26.12	7.74	4.68	0.0	-4.7
27.81	4.74	1.68	0.3	-0.51	27.81	-4.99	-8.05	265.2	8.1
28.1	-3.84	-6.90	119.4	2.98	28.1	8.24	5.18	0.0	-5.2
29.24	6.74	3.68	0.1	-1.11	29.24	6.81	3.75	0.1	-3.8
27.6	2.23	-0.83	1.8	0.25	27.6	-3.34	-6.40	84.6	6.4
29.14	8.08	5.01	0.0	-1.51	29.14	-2.60	-5.66	50.5	5.7
23.83	9.64	6.58	0.0	-1.98	23.83	4.94	1.88	0.3	-1.9
29.53	10.36	7.30	0.0	-2.20	29.53	11.00	7.94	0.0	-7.9
25.43	2.30	-0.76	1.7	0.23	25.43	-2.95	-6.01	64.4	6.0
26.41	10.13	7.07	0.0	-2.13	26.41	-4.94	-8.00	255.7	8.0
24.7	4.38	1.32	0.4	-0.40	24.7	3.56	0.50	0.7	-0.5
29.56	4.46	1.40	0.4	-0.42	29.56	4.72	1.66	0.3	-1.7
26.51	10.30	7.24	0.0	-2.18	26.51	6.01	2.94	0.1	-2.9
28.13	8.48	5.42	0.0	-1.63	28.13	8.53	5.47	0.0	-5.5
29.4	10.76	7.70	0.0	-2.32	27.75	0.06	-3.00	8.0	3.0
27.75	4.93	1.87	0.3	-0.56	24.18	3.29	0.23	0.9	-0.2
24.18	6.84	3.78	0.1	-1.14	29.18	7.35	4.29	0.1	-4.3
29.18	6.64	3.57	0.1	-1.08	26.92	8.27	5.21	0.0	-5.2
26.92	5.41	2.35	0.2	-0.71	29.08	5.54	2.48	0.2	-2.5
29.08	4.37	1.31	0.4	-0.39	26.16	2.62	-0.44	1.4	0.4
26.16	0.00	0.88	0.5	-0.27	25.63	5.64	2.58	0.2	-2.6

qRT-PCR data and primary calculation of miR-1247 in control and cancer patient

The primary calculation of miR-1247 expression level									
Patients					Control				
Ct	Δ ct	$\Delta\Delta$ ct	$2^{\Delta-\Delta\Delta$ ct	Log value	Ct	Δ ct	$\Delta\Delta$ ct	$2^{\Delta-\Delta\Delta$ ct	Log value
37.36	19.723	19.265	1.58701E-06	-19.26526	20.62	0.63	3.44	0.09	-1.04
21.21	5.396	4.939	0.032588855	-4.939477	20.62	-12.9	-14.47	22717.85	4.36
34.77	17.886	17.429	5.66677E-06	-17.42904	20.62	-5.68	-7.18	145.30	2.16
28.20	4.375	3.918	0.066132919	-3.918487	20.62	-3.25	-4.75	26.82	1.43
26.28	6.423	5.966	0.015999301	-5.965847	20.62	-2.56	-4.06	16.72	1.22
26.51	4.385	3.928	0.065701029	-3.927940	20.62	-5.92	-7.42	170.85	2.23
24.48	10.328	9.871	0.001067893	-9.871017	20.62	-6.30	-7.80	222.93	2.35
19.41	-0.820	-1.277	2.422736267	1.2766373	20.62	-12.8	-14.25	19439.16	4.29
24.34	2.295	1.838	0.279682351	-1.838138	20.62	-2.26	-3.76	13.53	1.13
35.22	1.143	0.686	0.621495252	-0.686184	20.62	1.10	-0.40	1.32	0.12
19.57	3.175	2.718	0.151970335	-2.718138	20.62	1.28	-0.22	1.17	0.07
29.69	15.858	15.401	2.31195E-05	-15.40053	20.62	-7.64	-9.14	565.82	2.75
20.59	3.132	2.675	0.156588124	-2.674953	20.62	2.66	1.16	0.45	-0.35
26.82	12.429	11.972	0.000248907	-11.97210	20.62	-12.6	-14.12	17830.40	4.25
30.70	2.677	2.220	0.214574662	-2.220448	20.62	2.06	0.56	0.68	-0.17
21.76	11.741	11.284	0.000400971	-11.284	20.62	-1.24	-2.74	6.66	0.82
25.69	11.976	11.519	0.000340739	-11.51904	20.62	2.89	1.39	0.38	-0.42
20.45	2.186	1.729	0.301703557	-1.728796	20.62	2.25	0.75	0.60	-0.22
35.12	9.586	9.129	0.001786415	-9.128716	20.62	0.76	-0.74	1.67	0.22
22.13	0.666	0.209	0.864914271	-0.209370	20.62	-1.80	-3.30	9.86	0.99
34.80	20.076	19.619	1.24159E-06	-19.61937	20.62	-10.3	-11.82	3609.79	3.56
21.66	3.988	3.531	0.086532516	-3.530613	20.62	-11.1	-12.61	6269.59	3.80
28.19	-0.099	-0.556	1.470572025	0.556377	20.62	1.74	0.24	0.85	-0.07
28.00	18.318	17.861	4.20155E-06	-17.8606	20.62	2.10	0.60	0.66	-0.18
26.57	0.685	0.228	0.853837376	-0.22796	20.62	-7.75	-9.25	610.51	2.79
31.91	11.762	11.305	0.000395346	-11.3045	20.62	-0.51	-2.01	4.04	0.61
28.31	-0.316	-0.773	1.708295335	0.772557	20.62	-4.21	-5.71	52.40	1.72
23.16	8.942	8.485	0.002790929	-8.485038	20.62	0.12	-1.38	2.60	0.42
33.32	5.466	5.009	0.031052039	-5.009168	20.62	1.03	-0.47	1.39	0.14
26.27	15.747	15.290	2.49543E-05	-15.29035	20.62	1.31	-0.19	1.14	0.06
26.01	4.303	3.846	0.06955052	-3.845794	20.62	-7.06	-8.56	378.72	2.58
32.97	6.196	5.739	0.018722938	-5.739049	20.62	-0.26	-1.76	3.40	0.53
25.48	16.541	16.084	1.44004E-05	-16.08352	20.62	-1.20	-2.70	6.51	0.81
25.63	7.142	6.685	0.009719579	-6.684890	20.62	1.97	0.47	0.72	-0.14
30.91	6.546	6.089	0.014686662	-6.089349	20.62	-2.92	-4.42	21.35	1.33
37.36	17.592	17.135	6.94604E-06	-17.13537	20.62	0.63	3.44	0.09	-1.04
21.21	-1.449	-1.906	3.747291391	1.9058481	20.62	-12.9	-14.47	22717.85	4.36
34.77	9.329	8.872	0.002134361	-8.871979	20.62	-5.68	-7.18	145.30	2.16
28.20	12.606	12.149	0.000220145	-19.26526	20.62	-3.25	-4.75	26.82	1.43
26.28	19.723	19.265	1.58701E-06	-4.939477	20.62	-2.56	-4.06	16.72	1.22

qRT-PCR data and primary calculation of miR-92a in control and cancer patient

The primary calculation of miR-92a expression level									
Patients					Control				
Ct	Δ ct	$\Delta\Delta$ ct	$2^{\Delta\Delta$ ct}	Log value	Ct	Δ ct	$\Delta\Delta$ ct	$2^{\Delta\Delta$ ct}	Log value
24.62	4.63	-3.86	14.48	3.86	37.36	17.37	3.08	0.00	-11.24
21.78	-4.52	-12.65	6422.12	12.65	34.77	8.47	1.00	0.00	-9.76
27.06	3.19	-4.61	24.35	4.61	28.20	4.33	11.38	2.60	1.38
22.70	-0.49	-8.34	323.61	8.34	26.28	3.09	-3.10	11.03	3.46
25.85	-0.69	-8.66	405.11	8.66	26.51	-0.03	15.77	0.20	-2.36
23.79	-3.13	-11.02	2074.75	11.02	24.48	-2.45	-2.29	48.13	5.59
23.99	1.11	-6.99	126.87	6.99	24.34	1.45	-1.07	30.36	4.92
24.30	4.78	-3.52	11.45	3.52	35.22	15.70	1.04	0.01	-7.56
27.66	8.31	0.33	0.79	-0.33	19.57	0.22	7.32	28.82	4.85
26.23	-2.04	-10.23	1204.60	10.23	29.69	1.42	15.06	0.05	-4.24
24.30	4.33	-3.67	12.73	3.67	20.59	0.63	2.48	39.99	5.32
27.33	-5.91	-14.16	18366.21	14.16	30.70	12.13	1.63	0.09	-3.49
24.25	5.68	-2.39	5.25	2.39	21.76	-0.10	-1.29	0.07	-3.90
22.18	0.32	-7.55	187.42	7.55	25.69	7.96	6.55	51.52	5.69
26.55	8.82	0.63	0.64	-0.63	20.45	2.07	4.67	0.38	-1.40
28.36	9.98	1.87	0.27	-1.87	35.12	15.26	-7.47	173.69	7.44
27.56	7.70	-0.87	1.83	0.87	22.13	-0.29	-3.13	23.99	4.58
23.00	0.58	-8.30	315.28	8.30	34.80	3.86	16.28	503.80	8.98
26.66	-4.29	-13.80	14305.52	13.80	28.19	9.31	14.86	0.00	-8.80
25.19	6.31	-2.53	5.79	2.53	18.00	-0.53	0.84	0.13	-2.92
23.05	4.53	-4.07	16.82	4.07	26.57	-1.80	4.80	482.34	8.91
26.33	-2.04	-11.45	2803.91	11.45	31.91	10.78	12.08	1.38	0.47
22.14	1.01	-8.45	349.58	8.45	28.31	3.48	-0.23	0.01	-6.29
27.68	2.85	-5.91	60.12	5.91	23.16	2.65	8.24	21.91	4.45
21.10	0.60	-8.71	420.09	8.71	33.32	13.73	7.47	8.68	3.12
28.16	8.56	-1.20	2.29	1.20	26.27	6.95	4.63	0.01	-6.78
27.66	8.34	-0.29	1.22	0.29	23.01	-4.68	2.72	2.80	1.49
23.08	-4.61	-13.53	11865.92	13.53	35.97	15.08	13.95	5.20	2.38
26.22	5.33	-4.19	18.20	4.19	21.48	-0.35	-1.43	0.00	-8.07
25.39	3.57	-3.26	9.59	3.26	25.63	6.98	2.80	310.33	8.28
23.01	4.35	-6.61	97.96	6.61	30.91	7.37	2.53	3.11	1.64

FORMS

ETHICS COMMITTEE DECISION



T.C.
İSTANBUL ÜNİVERSİTESİ
İSTANBUL TIP FAKÜLTESİ
KLİNİK ARAŞTIRMALAR ETİK KURULU



Sayı : 481

Tarih : 27.03.2018

Konu: Prof. Dr. Sadrettin PENÇE hk.

Sayın Prof. Dr. Sadrettin PENÇE
Deneysel Tıp Araştırma Enstitüsü

İlgi : Moleküler Tıp Anabilim Dalının 19/03/2018 gün ve 104563 sayılı yazısı

Sorumlu araştırmacılığını üstlendiğiniz ve Doktora Öğr. Amal SRGEWA' nın yürüteceği 2018/454 dosya numaralı "MikroRNA'ların (miR-92a, mir-195, mir-214, mir 224 ve mir 1247) düzeylerini prognostik ve değerlendirici faktör olarak Türk popülasyonunda değerlendirilmesi" (Evaluation of microRNAs (miR-92a, mir-195, mir-214, mir 224 and mir 1247) as predictive markers of colon rectal cancer (CRC) and/or prognostic factor in the Turkish population) başlıklı çalışma kurulumuzun 23/03/2018 tarih ve 06 sayılı toplantısında görüşülerek etik yönden uygun bulunmuş olup, tutanaklar ekte sunulmuştur.

Bilgilerinizi rica ederim.

Prof. Dr. A.Yağız ÜRESİN

İstanbul Tıp Fakültesi Klinik Araştırmalar
Etik Kurul Başkanı

Eki: İstanbul Tıp Fakültesi Klinik Araştırmaları Etik Kurulu Karar Formu

PATENT RIGHT



PLAGİARİSM RATE

Evaluation of microRNAs (miR-92a, miR-126, miR-195, miR-214, miR-224 and miR-1247), as predictive markers of Colorectal Cancer (CRC) and/or prognostic factor in Turkish population 2

ORIJİNALLIK RAPORU

%5	%3	%3	%3
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	www.ncbi.nlm.nih.gov İnternet Kaynağı	<%1
2	Submitted to Philadelphia College of Osteopathic Medicine Öğrenci Ödevi	<%1
3	Submitted to Kwame Nkrumah University of Science and Technology Öğrenci Ödevi	<%1
4	Wanpeng Yu, Xiao Liang, Xiangdong Li, Yuan Zhang, Zhenqing Sun, Ying Liu, Jianxun Wang. "MicroRNA-195: a review of its role in cancers", OncoTargets and Therapy, 2018 Yayın	<%1
5	www.cohesionbio.com İnternet Kaynağı	<%1
6	Cancer Diagnostics, 2004. Yayın	<%1

CURRICULUM VITAE

Personal information

Name	Amal	Surname	Srgewa
Place of birth	Libya	Birth date	03-06-1984
Email		Nationality	Libyan

Education level

	Name of the Institution	Graduation year
Doctoral	Istanbul University	2015-.....
Master	University of Sheffield	2011
Bachelor	Omar-Almukhtar University	2008
High school	Alzahra high school	2001

Work Experience

	Duty	Institution	Duration
1.	General practitioner	Al-whada hospital/pediatric department	2009 - 2010
2.	Tutor assistant	Medical technology collage	2009 - 2010
3.	Tutor	Medical faculty	2011- 2015

Languages	Understanding reading	Speaking	Writing
Arabic	Mother tongue		
English	Very good	Very good	Very good
Turkish	Good	Good	Good

*very good, good, poor

Computer Skills

Program	Ability to use
Microsoft	Very good
SPSS	Very good
Graph prizm	Very good

Research techniques skills

Molecular Genetic, PCR, Real Time-PCR, ELISA, Western Blot, Transwell migration and Invasion test, genetic material (RNA ve DNA) ve electrophoresis technique.

Special Interests (Hobbies): Reading

