T.C.

TOKAT GAZIOSMANPAŞA UNIVERSITY

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PHD DEGREE PROGRAM

INVESTIGATION OF THE ROLES OF SEVERAL MICRORNAS AND TARGET GENES IN COLORECTAL CANCER CHEMORESISTANCE

PHD THESIS

Ali Sedeeq NOAMAN

Advisor: Prof. Dr. Ercan ÇAÇAN

TOKAT-2025

ETHICS CONTRACT

According to the thesis writing guide of Tokat Gaziosmanpaşa University Graduate Education Institute, I declare that the PhD thesis titled "Investigation of the roles of several MicroRNAs and target genes in colorectal cancer chemoresistance", which I prepared under the supervision of Prof. Dr Ercan CACAN, is an original study following scientific ethical values and rules. I declare that it is and will accept all legal sanctions if the contrary is determined.

06 /10 /2025

Ali Sedeeq NOAMA

JÜRİ KABUL VE ONAY

Ali Sedeeq NOAMAN tarafından hazırlanan "**Investigation of the Roles of Several MicroRNAs and Target Genes in Colorectal Cancer Chemoresistance**" adlı tez çalışmasının savunma sınavı 17/09/2025 tarihinde yapılmış olup aşağıda verilen Jüri tarafından Oy Birliği ile Tokat Gaziosmanpaşa Üniversitesi Lisansüstü Eğitim Enstitüsü Biyoloji Anabilim Dalı'nda Doktora Tezi olarak kabul edilmiştir.

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ÖZET

BAZI MİKRORNA VE HEDEF GENLERİN KOLOREKETAL KANSER KEMODİRENCİ ÜZERİNDEKİ ROLLERİNİN ARAŞTIRILMASI

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Kolorektal kanser, dünya genelinde kanser kaynaklı ölümlerin önde gelen nedenlerinden biri olmaya devam etmekte olup, kemoterapiye direnç, tedavi sonuçlarını sınırlamaktadır. Bu tez, iki kemoterapötik ajan olan doksetaksel ve lapatinib'in antikanser etkilerinin moleküler mekanizmalarını araştırmakta ve bunların kolorektal kanser hücre hatlarındaki bazı hedef gen ve mikroRNA ekspresyonu üzerindeki etkilerini incelemektedir. Çalışmanın ilk bölümünde, bu ilaçların farklı kolorektal kanser hücre hatlarındaki sitotoksik etkileri değerlendirilmiş ve muamele sonrası hedef ilişkili genlerin ekspresyonu analiz edilmiştir. Her iki ajan da hücre canlılığını doz bağımlı şekilde anlamlı olarak azaltmıştır. Gen ekspresyon profillemesi, proliferasyonu, transkripsiyonel regülasyon ve onkogenez ile ilişkili genlerin down-regülasyonunu ve apoptosis ile hücre döngüsü ilişkili genlerin up-regülasyonunu gözlemlenmiştir. İlginç bir şekilde, bazı ilaç direnci ile ilişkili genler de indüklenmiş olup, sürdürülebilir terapötik etkinlik önünde potansiyel engelleri işaret etmektedir. Çalışmanın ikinci bölümünde, doksetaksel ve lapatinib'e yanıt olarak seçilmiş kanser ilişkili mikroRNA'ların modülasyonu incelenmiş ve miR-595'in onkogen E2F7 üzerindeki düzenleyici rolü araştırılmıştır. Yapılan uygulama birkaç mikroRNA'nın hücre hattına bağlı olarak farklı ekspresyon paternlerini değiştirmiştir. Özellikle, miR-595'in E2F7 ekspresyonunu negatif olarak düzenlediği ve bunun kemoterapi direncini etkileyebilecek fonksiyonel bir etkileşim önerdiği gözlemlenmiştir. Bu bulgular, doksetaksel ve lapatinib tarafından indüklenen moleküler yanıtlar hakkında yeni bilgiler sunmakta ve miR-595/E2F7 ekseninin hedeflenmesinin kolorektal kanserde kemoterapi etkinliğini artırabileceğini düşündürmektedir.

Anahtar Kelimeler: Kolorektal Kanser, Lapatinib, Dosetaksel, miRNA'lar, Tümör ilişkili genler

ABSTRACT

INVESTIGATION OF THE ROLES OF SEVERAL MICRORNAS AND TARGET GENES IN COLORECTAL CANCER CHEMORESISTANCE

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Colorectal cancer remains one of the leading causes of cancer-related mortality worldwide, and resistance to chemotherapy continues to limit therapeutic outcomes. This thesis investigates the molecular mechanisms underlying the anticancer effects of two chemotherapeutic agents, docetaxel and lapatinib, and explores their impact on gene and microRNA expression in colorectal cancer cell lines. In the first part of the study, we evaluated the cytotoxic effects of these drugs across different colorectal cancer cell lines and analyzed the expression of key cancer-related genes following treatment. Both agents significantly reduced cell viability in a dose-dependent manner. Gene expression profiling revealed downregulation of genes involved in proliferation, transcriptional regulation, and oncogenesis, alongside upregulation of genes linked to apoptosis and cell cycle arrest. Interestingly, some drug resistance–associated genes were also induced, highlighting potential barriers to sustained therapeutic efficacy.
In the second part, we examined the modulation of selected cancer-associated microRNAs in response to docetaxel and lapatinib and investigated the regulatory role of miR-595 on the oncogene E2F7. Treatment altered the expression patterns of several miRNAs in a cell line dependent manner. Notably, miR-595 negatively regulated E2F7 expression, suggesting a functional interaction that may influence chemoresistance. Collectively, these findings provide novel insights into the molecular responses induced by docetaxel and lapatinib and suggest that targeting the miR-595/E2F7 axis could enhance chemotherapy efficacy in colorectal cancer.

Keywords: Colorectal Cancer, Lapatinib, Locetaxel, miRNAs, Tumor-Associated Genes

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation	Explanation
1β	Interleukin-1 beta (often abbreviated as IL-1β)
13'- UTR	3' Untranslated Region
5′UTR	5' Untranslated Region
5-FU	5-Fluorouracil
A549 cells	Human lung carcinoma cell line
Apo-1	Apoptosis Antigen 1
ARC	Apoptosis Repressor with Caspase Recruitment Domain
ATP	Adenosine Triphosphate
CCNB1	Cyclin B1 gene
CCND1	Cyclin D1 gene
CD95	Cluster of Differentiation 95 (also known as Fas receptor)
cDNA	Complementary DNA
CHIP-seq	Chromatin Immunoprecipitation Sequencing
CRC	Colorectal Cancer
DD	Death Domain
DISC	Death-Inducing Signaling Complex
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
Doc	Docetaxel
DR	Death Receptor
DR4	Death Receptor 4
E2F	E2F Transcription Factor Family
EDTA	Ethylene diaminetetraacetic Acid
EGFR1/ErbB1	Epidermal Growth Factor Receptor 1
EHF	Ets Homologous Factor
EMEM	Eagle's Minimum Essential Medium
EOC	Epithelial Ovarian Cancer
ErbB2	Epidermal Growth Factor Receptor 2 (HER2)

ERK kinase	Extracellular Signal-Regulated Kinase
ESCC	Esophageal Squamous Cell Carcinoma
FBS	Fetal Bovine Serum
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
GC	Gastric Cancer
GLOBOCAN	Global Cancer Observatory
H358 cells	Human non-small cell lung cancer cell line
HCC	Hepatocellular Carcinoma
HER	Human Epidermal Growth Factor Receptor
HT29	Human colorectal adenocarcinoma cell line
IC50	Inhibitory Concentration 50
IL	Interleukins
JNK	c-Jun N-terminal Kinase
Lap	Lapatinib
let-7	Let-7 microRNA family
lin- 14, lin28, lin-41, lin-42	Genes related to the microRNA pathway in *Caenorhabditis elegans*
MAPK	Mitogen-Activated Protein Kinase
MDR	Multidrug Resistance
MEKK	Mitogen-Activated Protein Kinase Kinase Kinase
mFasL	Membrane-bound Fas Ligand
microRNAs	Small, non-coding RNA molecules
miRNAs	Small, non-coding RNA molecules
mRNA	Messenger RNA
MTT	Methylthiazolyldiphenyl-Tetrazolium Bromide
MYC	MYC proto-oncogene
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
Ng	Nanogram
NH4+	Ammonium Ion
NSCLC	Non-Small Cell Lung Carcinoma

p53	Tumor Suppressor Protein p53
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PI3K/AKT	Phosphatidylinositol 3-Kinase/Protein Kinase B pathway
PKHD	Polycystic Kidney and Hepatic Disease 1 (involved in fibrosis)
PPE	Personal Protective Equipment
Pri-miRNA	Primary microRNA
PTC	Papillary Thyroid Cancer
PTEN	Phosphatase and Tensin Homolog
QIAzol	Reagent used for RNA extraction
qRT-PCR	Quantitative Real-Time Polymerase Chain Reaction
RAGE	Receptor for Advanced Glycation End Products
RanGTP	Ras-Related Nuclear Protein bound to GTP
RISC	RNA-Induced Silencing Complex
RNA	Ribonucleic Acid
RNase-free	Free of Ribonuclease enzyme
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SCC	Squamous Cell Carcinoma
SEM	Scanning Electron Microscopy
SOX7	SRY-Box Transcription Factor 7
SW-620	Human colorectal adenocarcinoma cell line
TGF	Transforming Growth Factor
TKI	Tyrosine Kinase Inhibitor
TM-RNA	Transfer-messenger RNA
TNF	Tumor Necrosis Factor
TNFRSF6	Tumor Necrosis Factor Receptor Superfamily Member 6
TP53	Tumor Protein p53 gene
TRAIL	Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand
TRAIL-R1	Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Receptor 1
UV	Ultraviolet Light
VEGF	Vascular Endothelial Growth Factor

WNT1	Wingless-Type MMTV Integration Site Family, Member 1
µL	Microliter

1. INTRODUCTION

Colorectal cancer (CRC) is one of the most prevalent malignancies globally and remains a significant cause of cancer-related mortality. According to the World Health Organization, CRC is the third most diagnosed cancer and is the second leading cause of cancer-related deaths globally (Brett et al., 2018). Despite advances in early detection and chemotherapy, the 5-year survival rate for people with advanced CRC remains poor. Resistance to chemotherapy is a significant obstacle in CRC treatment. It greatly leads to therapy failure and disease progression (Zhao et al., 2021).

Chemoresistance in CRC can be categorized as intrinsic, meaning that the tumor is resistant to therapy from the first, or acquired, which occurs during or after treatment. The complex and multifactorial molecular basis of chemoresistance. These mechanisms consist in elevated drug efflux, apoptosis suppression, more efficient DNA repair, epithelial to mesenchymal transition (EMT), cancer stem cells (Holohan et al., 2013). Mechanisms underlying these effects need to be further elucidated to optimize therapeutic responses and circumvent resistance.

miRNAs have been considered as important post-transcriptional suppressors of gene expression of various biological processes, such as tumorigenesis and drug resistance in recent years. miRNAs are small (18–25 nt) non-coding RNAs that base pair with complementary sequences in the 3′ untranslated regions (3′UTRs) of target mRNAs, which result in mRNA degradation or repression of translation (Bartel, 2009). miRNAs are involved in a large number of processes in various types of cancer, including CRC. Disordered expression of miRNAs could lead to the development of tumors, metastasis, and drug resistance (Garzon et al., 2009). Several studies have diagnosed particular miRNAs that play vital roles in modulating chemosensitivity in CRC. For example, miR-21 is frequently overexpressed in CRC and has been proven to promote resistance to 5-fluorouracil (5-FU) via concentrated on the tumor suppressor gene, PTEN and the DNA mismatch repair gene, hMSH2 (Valeri et al., 2010). Similarly, downregulation of miR-34a is related to resistance to oxaliplatin with the aid of allowing survival of cancer stem

cells (Siemens et al., 2013). These miRNAs can act as both oncogenes (OncomiRs) or tumor suppressors relying on their targets and expression patterns.

2. LITERATURE REVIEW

2.1. Cancer and the Purpose of Cancer Research

Cancer is characterized by the aberrant proliferation of cellular populations within a specific tissue, resulting in the disruption of the regular cell cycle. It is one of the top causes of death, particularly in developing countries, which account for 82% of the world's population. According to the article "Global Cancer Statistics" published in 2018, the estimations provided by GLOBOCAN indicate that there were around 18.1 million new cases of cancer and 9.5 million deaths globally. In the last few decades, the majority of new cancer diagnoses and fatalities have been concentrated in developing nations. (Lindsey et al., 2012), In light of this load and these deaths, the findings of this study suggest that lowering mortality rates through earlier disease diagnosis is not only a humanitarian obligation but will also help us become more conscious of our conscientious responsibilities.

2.2. Roles of MicroRNAs in Cancer

In 1993, Caenorhabditis elegans yielded the first miRNA, lin-4, It was discovered to have a role in regulating the development of larvae by suppressing a nuclear protein encoded by lin-14. Let-7, the second discovered miRNA, connects to the 3′ UTR of numerous genes, including lin-14, Oncogene 2, lin-28, lin-41, lin-42, and DAF-12, and is produced late in development. Following the identification of lin-4 and let-7, miRNAs in a variety of species, including both mammals and plants, were discovered (Brennecke J et al., 2003; Lagos-Quintana M et al., 2002). The maturation of microRNA occurs in three stages: Pri-miRNA transcription, cleavage of pre-miRNA in the nucleus and the last cleavage to create mature microRNA occur in the cytoplasm (Meister G et al.,2004). Pri-miRNA is produced by RNA polymerase II, the length can be set to 1 kb, and it forms hairpin loops from DNA, Pri-miRNAs can be discovered as free transcripts or inside the introns of other genes (Lee RC et al., 1993). RNase Drosha cleaves the pri-miRNA after transcription to create a precursor microRNA (pre-miRNA) with a length of 60–70 bp (Bartel DP et al., 2004). The transport of miRNA from the nucleus to the cytoplasm was suspected but remained obscure until 2003 when two independent groups published their discoveries that the transporter is RanGTP dependent1Exportin 5 (Yi R, et 1al., 2003; Bohnsack MT et al., 2001). After that Dicer

cleaves pre-miRNA on the loop end in the cytoplasm to create a miRNA duplex. A helicase then unwinds the duplex to create two mature microRNAs, either one or both of which may be active (Hutva Wagner et al., 2001). The mature microRNAs suppress the production of proteins, they target and cleave mRNA via the RNA-induced silencing complex (RISC) (Hammond SM et al., 2000).

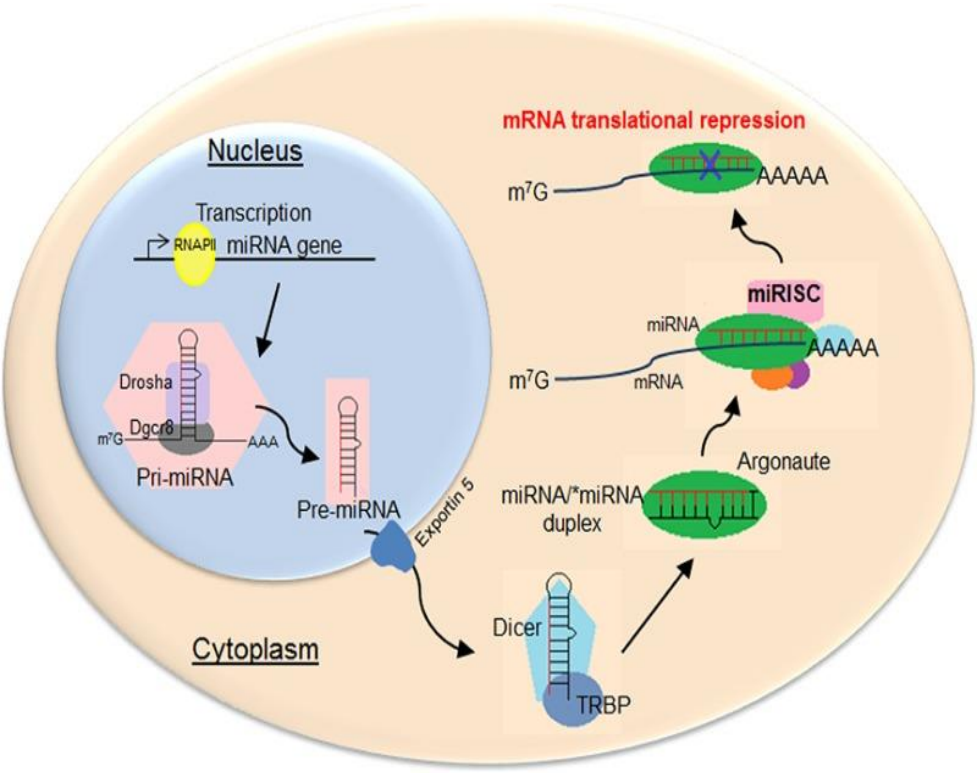

Figure 2.1. Schematic of miRNA biogenesis and function, miRNA biogenesis begins in the nucleus and transported the cytosol out by Exportin 5 (Hajarnis S et al., 2015).

2.3. Colorectal Cancer Overview

Colorectal cancer (CRC) is one of the most common types of cancer worldwide, affecting both males and females, the prognosis for advanced cases is poor, with a five-year survival rate of less than 40% in advanced stages (Pohl et al., 2000). Approximately 75% of newly diagnosed CRC cases occur in individuals without any known risk factors, while 15% to 20% of cases are found in patients without a genetic abnormality but with a family history of the disease. Hereditary nonpolyposis

accounts for about 5% of CRC cases, and familial adenomatous polyposis causes 1% of cases (Winawer SJ et al., 1997). Identifying new oncogenes and tumor suppressors could enhance our understanding of CRC biology and lead to potential new therapeutic targets (Kitahara et al., 2001).

Early detection of CRC is critical, and finding novel non-invasive biomarkers that can identify tumors in the early stages is of utmost importance (Mara Marcuelloa et al., 2019). Reliable diagnostic approaches, including biopsy-based procedures, colonoscopy, histological examination of tumor cells, and imaging techniques such as x-rays and magnetic resonance, have been the cornerstone of CRC therapy for many years.

Since the introduction of chemotherapy in 1957, the survival rate for CRC patients has improved (Astrup, G. L et al., 2017; Basch, E et al., 2016). Over the last several decades, advancements in chemotherapy agents, surgical techniques, screening, and radiotherapy have further increased survival rates (Basch, E et al., 2016; Benitez Majano, S et al., 2019). However, despite these improvements, the disease and its treatment significantly impact the quality of life and daily functioning of CRC patients and their families. The complex health situation and unpredictable nature of living with a CRC diagnosis present challenge for patients' social networks and environments (Foltran, L et al., 2014).

The continuous increase in CRC prevalence, particularly in developing countries, is linked to population aging and unhealthy, westernized lifestyles, including diets low in fresh fruits and vegetables and high in processed foods, fats, and red meat, along with a lack of physical activity (Jemal A et al., 2011). Lifestyle factors contribute to approximately 57.2% and 50.2% of incident CRC cases in men and women, respectively, regardless of age (Islami F et al., 2018) The World Cancer Research Fund/American Institute for Cancer Research lists processed and red meat, alcoholic beverages, and obesity as significant risk factors for CRC (Larsson SC et al., 2006; Pan SY et al., 2009). Therefore, there is a substantial opportunity to reduce CRC risk through lifestyle modifications. Colonoscopy remains an effective screening method, providing long-term protection against CRC occurrence and death by detecting and removing adenomas and enabling earlier diagnosis (Nishihara R et al., 2013; Holme Øet al., 2013).

2.3.1. Epidemiology of colorectal cancer

According to the findings of GLOBOCAN 2018, cancer of the colon is the fourth most incidence type of cancer in the globe, whilst cancer of the rectum is the eighth most incident type of cancer. CRC collectively account for 11% of all cancer diagnoses worldwide, making them the third most common form of cancer to be identified and treated (Bray F et al., 2018).

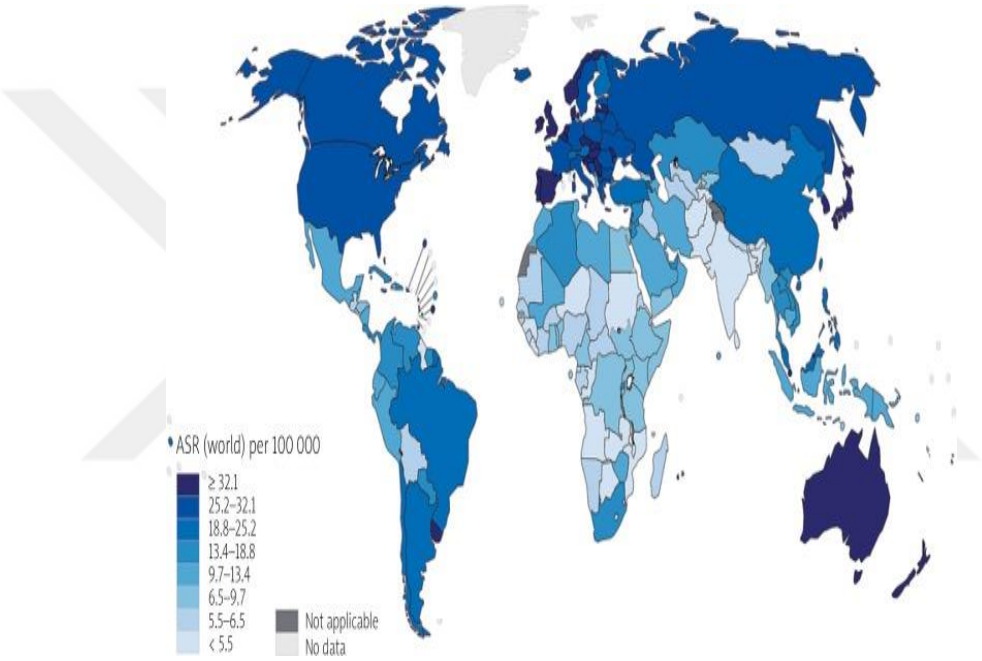

Figure 2.2. This map displays the estimated age-standardized incidence rates (worldwide) for colon cancer in 2018, divided into sexes and age groups for the colorectum (Bray F. et al., 2018).

In the year 2018, it predicts that approximately 704,000 new instances of rectal cancer would be diagnosed, while approximately 1,096,000 new cases of colon cancer will be identified. Collectively, these figures encompass a total of 1.8 million newly diagnosed cases of colorectal cancer its stands as the most frequently diagnosed malignancy among the male population in a notable proportion of 10 out of the 191 countries across the globe. Colorectal cancer does not emerge as the most frequently diagnosed cancer among women in any country (Bray F et al., 2018).

Colorectal cancer exhibits a higher incidence rate in the male population compared to females, with a prevalence that is approximately 3-4 times greater in developed countries when compared to developing nations. Worldwide, colorectal cancer is more common in men than women, with an incidence of 23.6 per 100,000 males and 16.3 per 100,000 females (Ferlay et al., 2018).

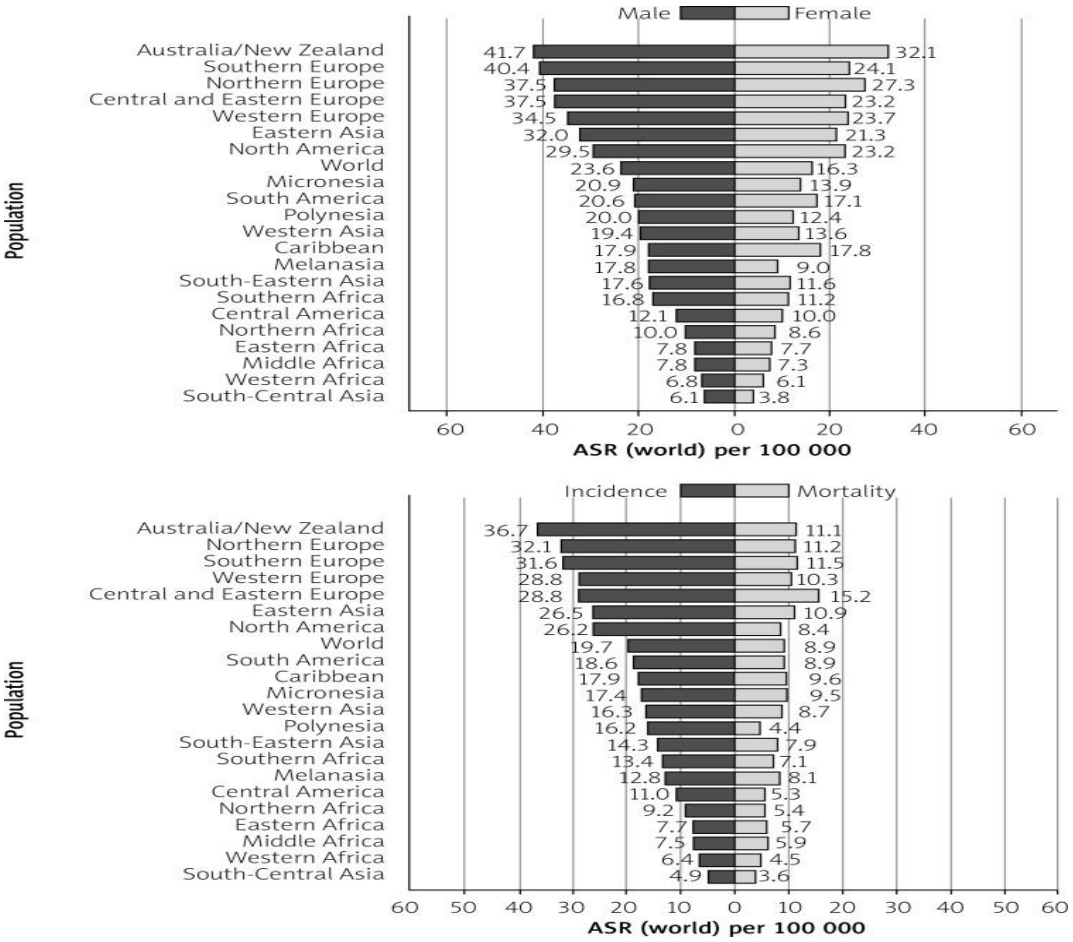

Figure 2.3. Global age-standardized cancer female male and incidence and mortality rates by region and gender.

The provided visual representation shows a bar chart illustrating the age-standardized (world) incidence rates of colorectal cancer in the year 2018. the chart presents the incidence rates categorized by sex, along with the age-standardized (world) incidence and mortality rates for colorectal cancer in the same year. (Ferlay J et al.,2018). In the year 2018, it is projected that approximately 576,000 individuals of the male gender and 521,000 individuals of the female gender will receive a diagnosis of colon cancer. The

above occurrence signifies a cumulative risk of 1.51% for colon cancer in males aged 0-74 years, while females face a slightly lower risk of 1.12%. It is projected that approximately 430,000 individuals of the male gender and 274,000 individuals of the female gender will receive a diagnosis of rectal cancer. The cumulative, lifetime risks for the aforementioned conditions are estimated to be 1.2% and 0.65%, respectively (Bray F et al., 2018).

Colon and rectal cancer pose the greatest danger to individuals residing in developed nations. The regions with the highest incidence of colon cancer include Southern Europe, Australia/New Zealand, and Northern Europe. The regions associated with rectal cancer include Eastern Europe, Australia/New Zealand, and Eastern Asia. North America is also recognized for having some of the highest rates of incidence for both types of cancer. The nation with the highest rate of CRC per 100,000 people is Norway (29.3) for women and Hungary (70.6) for men. Men are most1commonly diagnosed with colorectal cancer (CRC) in Japan, South Korea, Saudi Arabia, Oman, Yemen, UAE, Bahrain, Qatar, Kuwait, and Slovakia. On the other hand, Southern Asia and all of Africa have the lowest incidence rates of cancer in both sexes (Bray F et al., 2018).

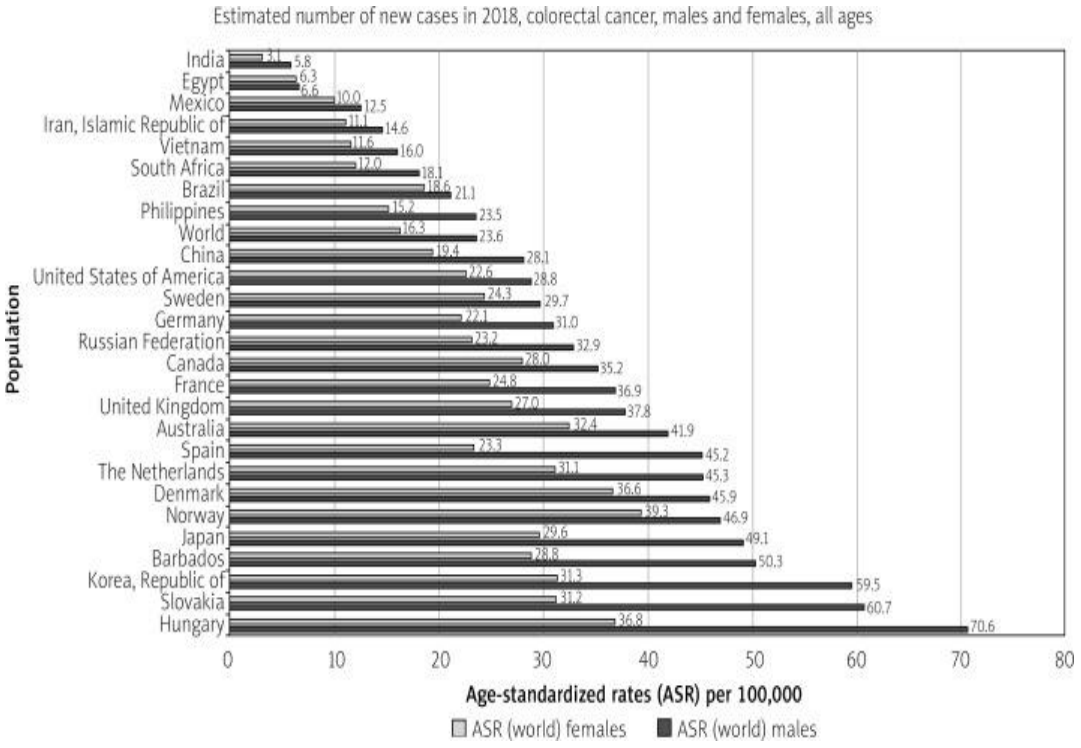

Figure 2.4. Bar chart showing country-specific standardized (world) incidence rates of new colorectal cancer cases by sex in 2018 (Ferlay J et al., 2018).

Overall, CRC incidence is highly variable by region, with up to eight-fold variations between countries. In countries undergoing a major developmental transition, incidence rates tend to rise uniformly with increasing HDI, suggesting a causal relationship (Bray F et al., 2018).

2.3.2. Etiology colorectal cancer

The CRC usually begins with the non-cancerous proliferation of mucosal epithelial cells. These growths are known as polyps and can grow gradually for 10–20 years before becoming cancerous. The most common form is an adenoma or polyp that originated from granular cells, whose function is to produce the mucus that lines the large intestine (Stryker SJ et al., 1987) Only about 10% of all adenomas progress to invasive cancer, although the risk of cancer increases as the polyp grows larger. Invasive cancer arising from such polyps is known as adenocarcinoma and accounts for 96% of all CRCs (Stewart SL et al., 2006) Colorectal cancer that grows into the wall of the colon or rectum can penetrate blood or lymphatic vessels, allowing metastasis to distant organs via the blood or to nearby lymph nodes. The extent of invasion determines the staging, and thus the prognosis, of a CRC diagnosis (Compton et al., 2000). In situ cancers are polyps that have not yet invaded the colon or rectum wall and are thus not reported as CRCs. Local cancers are those that have grown into the wall but have not yet extended past it. Regional cancers are those that have invaded nearby lymph nodes or tissues, while distant cancers are those that have metastasized, via the bloodstream, to distant organs with capillary beds where they have taken root, such as in the lungs or liver (Edge et al., 2010). Certain dietary and lifestyle choices can promote intestinal inflammation and modify the intestinal microflora to promote an immune response, both of which can facilitate polyp growth and conversion to cancer (Chan et al., 2019; Garrett, 2015).

2.4.MicroRNA and their Role in Cancer

2.4.1. Impact of miRNA dysregulation in cancer

To find appropriate biomarkers for colorectal cancer, numerous studies have examined the expression profiles of miRNAs in tissue, serum, and cell samples of patients. The high death rate is largely a result of cancer being discovered at an advanced stage of development. Therefore, it is important to discover suitable biomarkers for the early detection of colorectal cancer. Based on the literature review, we observed several types of miRNAs that show different expression profiles in various cancer types, and we aim to determine the expression profiles of these miRNAs in colorectal cancer. For example, miRNA-718 was first discovered in 2006, but it gained attention in 2014 when it was shown to repress VEGF (Vascular Endothelial Growth Factor) and decrease the growth of ovarian cancer cells (Wang X. et al., 2018). miR-718 plays a critical role in the development of several cancers and in tumorigenesis. However, miR-718's expression pattern, specific roles, and mechanisms of action in non-small cell lung cancer (NSCLC) remain unclear (Shu Wan et al., 2020). Its expression in NSCLC tissues and cell lines was assessed, and its clinical importance was evaluated among NSCLC patients. Furthermore, miR-718 was reported to influence the cancerous characteristics of NSCLC cells (Shu Wang et al., 2020). In squamous cell carcinoma of the esophagus (ESCC), patients had downregulated levels of plasma miRNA-718, which could be used as a diagnostic marker (Li Suna et al., 2016). Since miRNA-718 has not yet been investigated in colorectal cancer but has been studied in other types of cancer, we intend to examine its expression in CRC to determine whether it is up- or downregulated.

Another type of miRNA, miRNA-595, is downregulated in hepatocellular carcinoma tissues and cells and has been closely associated with poor overall survival in patients (Wang H. et al., 2020). Further analysis of miR-1199-5p expression in various human breast cancer cell lines demonstrated that epithelial cells show higher expression compared to breast cancer mesenchymal cells (Maren Diepenbruck et al., 2017). It has been further demonstrated that miR-1199-5p downregulation is required for lung

metastasis in vivo, and it influences cell differentiation, migration, and invasion of mesenchymal tumor cells (Maren Diepenbruck et al., 2018). miR-185-5p has been reported to be overexpressed and can serve as a biomarker for hepatocellular carcinoma (Junyan Tao et al., 2017). Additionally, the expression of miRNA-206 has been reported to be significantly downregulated in CRC patients compared to healthy controls (Xingcun Liu et al., 2017). Based on the most recent studies, miRNAs are being proposed as promising biomarkers for CRC prognosis, diagnosis, and response to therapy (Burcin Baran et al., 2018). According to other studies, patients with various types of malignancies can also be identified through the dysregulation of miRNAs in their circulation (Lodes M. et al., 2009; Heneghan et al., 2010).

miRNAs function as either tumor suppressors or oncogenes in carcinogenesis. Through their promoter regions, transcription factors such as p53 or MYC can activate or inhibit miRNA expression. One of the first tumor suppressor miRNAs to be identified, miR-145, has been shown to be downregulated in numerous cancers, including breast and colon carcinomas (Shi B. et al., 2009; Wang S. et al., 2009). Changes in TP53 expression levels influence the expression of miR-145, which is found to be downregulated in many CRC patients. Without a doubt, the genetic context plays an essential role in influencing miRNA expression in CRC (Yang L. et al., 2009; Michael MZ et al., 2003). Specific miRNAs are expressed at higher levels when 5-fluorouracil (5-FU) is administered to wild-type HCT-116 cells, and nearly all of these miRNAs have binding sites for the TP53 gene. These miRNAs are not upregulated when 5-FU is applied to HCT-116 cells with the p53 gene knocked out (Matsumoto K. et al., 2014). Numerous studies have identified a variety of gene sets as well as gene expression profiles that can be used to classify and understand this life-threatening condition (R. Liu et al., 2017).

2.5. Target MicroRNAs Profiling

miR-718 has been identified as an anti-oncogenic biomarker in hepatocellular carcinoma (HCC). miR-718 expression plays a significant role in regulating Papillary Thyroid Carcinoma cell growth, proliferation , metastatic ability, and metabolism (Wang X et al., 2018). miRNA-718 in human cancers. Previous studies have demonstrated that circulating miRNA-718 could be detectable in breast cancer and hepatocellular carcinoma, playing as a potentially predictive and prognostic biomarker. the role of miRNA-718 in esophageal squamous cell carcinoma ESCC is downregulated in patients and might serve as a potential diagnostic marker (Sun L et al., 2018).

Furthermore miR-595, a relatively recently characterized microRNA, has gained considerable attention for its regulatory roles in cancer biology. miR-595 are non-coding RNA molecules that regulate gene expression post-transcriptionally by binding to complementary regions in mRNA, leading to mRNA degradation or suppression. This regulatory mechanism allows miRNAs to influence cellular processes such as cell cycle control, proliferation, apoptosis, and differentiation, all of which are crucial in cancer progression (Han et al., 2021). miR-595 has shown varied roles in different cancers, acting as both a tumor suppressor and, in certain contexts, as an oncogene. For instance, in colorectal cancer, miR-595 targets oncogenes like E2F7, potentially increasing the sensitivity of cancer cells to chemotherapeutic agents and thereby acting as a tumor suppressor (Xie et al., 2023). Conversely, in glioblastoma, miR-595 has been reported to promote carcinogenesis by inhibiting SOX7 expression, highlighting its context-dependent effects (Hao et al., 2016). In ovarian cancer, miR-595 is downregulated, which enhances sensitivity to cisplatin and suggests a tumor-suppressive role. Similarly, in hepatocellular carcinoma, downregulation of miR-595 suppresses cell proliferation and metastasis by inactivating the NF-κB pathway, both in vitro and in vivo (Wang H et al., 2021). This dual capability acting as either a tumor suppressor or promoter depending on cellular environment and targets underlines miR-595's therapeutic potential in precision oncology. Researchers are exploring synthetic miRNA mimics to restore its tumor-suppressive effects or inhibitors to block its oncogenic actions. Although promising, further research is needed to fully map miR-595's molecular

targets and refine its potential application in targeted cancer therapies, especially to combat drug resistance and improve patient outcomes (Zhou et al., 2023; Li et al., 2021).

Moreover, miRNAs are critical regulators in cancer biology due to their influence on cell proliferation, metastasis, and apoptosis. Among these, miR-1199 has gained attention for its potential tumor-suppressive roles in several cancer types, influencing pathways that drive oncogenesis. Recent research has shown that miR-1199 can inhibit epithelial-mesenchymal transition (EMT), a key process in cancer metastasis, by downregulating. These transcription factors are heavily implicated in the invasion and spread of cancer cells, especially in aggressive malignancies such as breast cancer and lung cancer (Diepenbruck, M et al., 2017).

In colorectal cancer (CRC), miR-1199 has been shown to suppress the Wnt/β-catenin signaling pathway, which is a critical driver of tumor growth and progression in CRC. This pathway's inhibition by miR-1199 decreases cell proliferation and reduces metastatic capabilities. It has also been linked to the regulation of cell cycle genes like CCND1 and CDK6, further emphasizing miR-1199's tumor-suppressive function. The potential of miR-1199 as a therapeutic target is especially notable in CRC, given that activation of Wnt/β-catenin is a common feature of treatment-resistant CRC cases (Jiang et al., 2023; Chen Y et al., 20244).
In lung cancer, miR-1199 exerts its effects through downregulation of the PI3K/AKT pathway. This pathway is essential for cellular survival and metastasis, and its inhibition by miR-1199 leads to decreased viability and invasiveness of lung cancer cells. Such findings underscore miR-1199 has broader role across multiple cancer types, marking it as a versatile target in cancer therapy. Its ability to modulate pathways central to tumor growth and metastasis, like PI3K/AKT and Wnt/β-catenin, suggests that miR-1199-based therapies could complement existing treatments by targeting drug-resistant cell populations and reducing metastatic potential (Gao, S et al., 2021; Zhang et al., 2023).

As a therapeutic agent, miR-1199 demonstrates potential for incorporation into miRNA replacement therapies. By reintroducing or mimicking miR-1199 in tumor cells, these therapies could restore its regulatory role, reversing EMT and other tumor-promoting

pathways. Further preclinical studies will be essential to evaluate its efficacy and safety as part of therapeutic regimens, particularly in cases where standard treatments are insufficient (Kota et al., 2023; Zhang et al., 2023).

In addition, miR-185 has been found frequently to vary in abundance in samples from cancer patients compared to samples from healthy donors. The gene is located at chromosome 22q11.21.the miR precursor consists of 82 nucleotides and is the source of the two mature molecules miR-185-5p and miR-185-3p; according to available data, miR-185-5p is the predominantly produced molecule (Babaeenezhad E et al., 2022).

In recent years, evidence has accumulated that miR-185 is implicated in the pathogenesis of this group of highly malignant tumors. Its expression variations correlate with clinical features, such as tumor size, lymph node metastasis, tumor node metastatic stage, survival, recurrence and response to adjuvant therapy, and have diagnostic and prognostic potential. Interestingly, miR-185 is apparently involved in regulating both tumor suppressive and oncogenic processes (Babaeenezhad E et al., 2022).

Recent reports have shown that miRNA-185 plays critical roles in tumorigenesis and tumor progression in multiple human cancers, such as melanoma (He et al., 2017), ovarian cancer (Imam et al., 2010), colorectal cancer. However, the exact function of miR-185 in colon cancer remains unclear yet (Zhang W et al., 2018).

Finally, miR-206 is a member of the miR-1 family. The gene encoding this miRNA is located between the IL-17 and PKHD1 genes in human. The cytogenetic band of is 6p12.2. this miRNA has been shown to participate in the pathogenesis of a variety of malignant and non-malignant conditions. Like other member of this miRNA family, miR-206 has physiological roles as well miR-595 (Khalili an S et al., 2021).

miR-206 is known to regulate cell proliferation and migration and is involved in various types of cancer miR-206 It has been documented that downregulation of miR-206 is significant in human gastric cancer (GC), whereas its role in GC cell biological behaviors, including proliferation, migration, and invasion, has not been thoroughly investigated. MiR-206 levels have a negative association with lymph node metastasis

and tumor invasion, and patients with higher miR-206 expression have better prognoses. Functional studies demonstrated that miR-206 overexpression significantly suppresses GC cell proliferation, migration, and1invasion, and induces apoptosis in vitro (Deng M et al., 2019).

2.6. Cell Lines Utilized in this Study

The human colon adenocarcinoma cell line HT-29 was isolated from a primary tumor of a 44 years old Caucasian female in 1964 by Fogh and Trempe (1975). Since then, many cell lines have been derived from human colon cancers. Initially, this cell line was used to study different aspects of the biology of human cancers. However, these cells have attracted attention due to the fact they were able to express characteristics of mature intestinal cells, such as enterocytes or mucus producing cells (Martínez-Maqueda et al., 2015)

These cells have shown a high rate of glucose consumption and therefore, they require high glucose concentration in the medium. Under these standard growth conditions, i.e., in the presence of 25 mM glucose and 10 % serum, these cells display an undifferentiated phenotype; they grow as a multilayer of unpolarized undifferentiated cells, and functionally they do not express any typical characteristics of intestinal epithelial cells, and they express low amounts of hydrolases. Since the discovery that the replacement of glucose by galactose in the culture medium induced a reversible enterocytic differentiation (Pinto et al., 1982)

The factors secreted from cells in culture medium include metabolites, cytokines, growth factors, which are known to promote cell survival. Recently, the soluble factors secreted by HT-29 and other tumor cells in basal conditions and after gamma adiation have been reported. HT-29 secreted pro-inflammatory cytokines, such as, tumor necrosis factor (TNF) α and interleukins (IL) 1β and IL 6; growth factors such as platelet-derived growth factor AA and transforming growth factors (TGF) α and β; chemokines such as fractalkine, IL-8, monocyte chemoattractant protein-1 and interferon-γ-induced protein 10; pro-angiogenic factors such as IL-15 and vascular endothelial growth factor; and immune-modulatory cytokines such as

granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor and IL-3. It was suggested, based on previous reports on biopsy samples, that in vivo, a similar cytokine secretion profile can be observed (Desai et al., 2013).

Similarly, HCT-116 is a human colorectal carcinoma cell line widely used as a model system for studying colorectal cancer (CRC). This cell line was derived from the colon of an adult male and displays an epithelial morphology. HCT-116 is characterized by a mutation in the KRAS proto-oncogene at codon 13 (G13D), which plays a critical role in colorectal tumorigenesis by driving uncontrolled cell proliferation and resistance to apoptosis. The cell line is also mismatching repair (MMR)-deficient due to mutations in the MLH1 gene, a hallmark feature in a subset of CRC tumors associated with microsatellite instability (MSI). These genetic attributes make HCT-116 an excellent model for investigating the molecular mechanisms of MSI-positive CRC and its implications for therapeutic response (Ahmed et al., 2013; Bläker et al., 2004).

HCT-116 cells are particularly valuable for studying drug resistance in CRC, as they exhibit inherent resistance to certain chemotherapeutic agents, including 5-fluorouracil (5-FU) and oxaliplatin. This resistance has been linked to their unique genetic and molecular profile, including mutations in KRAS and altered apoptotic pathways. Researchers have used HCT-116 cells to explore strategies for overcoming chemoresistance, such as the combination of targeted therapies with conventional chemotherapy. For example, the use of small-molecule inhibitors targeting the PI3K/AKT/mTOR pathway has shown promise in sensitizing HCT-116 cells to chemotherapy, providing insights into potential therapeutic approaches (Ning et al., 2023).

In addition to their use in in vitro studies, HCT-116 cells are frequently employed in in vivo xenograft models. When injected subcutaneously or orthotopically into immunodeficient mice, HCT-116 cells reliably form tumors, enabling researchers to study tumor growth dynamics, angiogenesis, and metastatic behavior. Notably, HCT-116 xenografts have been instrumental in evaluating the efficacy of novel anticancer agents, including monoclonal antibodies, small-molecule inhibitors, and immune checkpoint blockers. These models also allow for the investigation of the tumor microenvironment, including1the role of immune cells and stromal components in cancer progression and

therapeutic response (Hollingsworth et al., 2015).

Recent studies have also utilized HCT-116 cells to explore the role of non-coding RNAs, particularly miRNAs in CRC biology. Dysregulation of specific miRNAs, such as miR-21, miR- 135b, and miR-206, has been observed in HCT-116 cells, implicating these molecules in processes such as proliferation, invasion, and resistance to therapy. Functional studies using miRNA mimics and inhibitors in HCT116 cells have provided valuable insights into the regulatory networks driving CRC progression and have identified potential therapeutic targets (Nielsen et al., 2017).

Finaly SW-620 is a human colorectal adenocarcinoma cell line derived from a lymph node metastasis of a primary colon carcinoma in a 51-year-old male patient. This cell line is commonly used as a model to investigate colorectal cancer (CRC), particularly in studies of metastasis and tumor progression. SW-620 cells are characterized by their aggressive growth behavior, high migratory capacity, and metastatic potential, making them a valuable tool for exploring the molecular mechanisms underlying CRC metastasis (Ahmed et al., 2013; Chen et al., 2015).

SW-620 cells exhibit genetic alterations typical of advanced CRC, such as mutations in the KRAS and TP53 genes. The presence of these mutations renders the cell line suitable for evaluating how these genetic aberrations influence tumor behavior, chemoresistance, and responses to targeted therapies. Additionally, SW620 cells have microsatellite stability (MSS), which differentiates them from other CRC cell lines like HCT116, enabling researchers to study molecular differences between MSS and microsatellite instability (MSI) CRC types (Shirasawa et al., 1993).

One of the unique aspects of SW-620 cells is their use in metastasis research. When compared to the SW480 cell line, which is derived from the primary tumor of the same patient, SW-620 cells exhibit distinct gene expression profiles and phenotypic characteristics associated with metastatic potential. This pair of cell lines serves as a robust model system for studying the transition from primary tumors to metastatic disease and identifying genes and signaling pathways that drive metastasis (Cheng et al., 2020).

In therapeutic research, SW-620 cells are extensively used to evaluate the efficacy of

chemotherapeutic agents and combination therapies. They are particularly relevant for studies on resistance mechanisms to drugs like 5-fluorouracil (5-FU), oxaliplatin, and irinotecan. For example, SW-620 cells have been instrumental in exploring the role of apoptotic pathways, including the DR4 (death receptor 4) and FAS-mediated signaling, in overcoming drug resistance. Additionally, these cells are a critical model for investigating the role of non-coding RNAs, such as (miRNAs), in CRC. Dysregulated miRNAs, including miR-21 and miR-135b, have been shown to modulate chemoresistance and metastatic behaviors in SW620 cells, providing insights into novel therapeutic targets (Nielsen et al., 2017).

2.7. Several Target Tumor Suppressor and Oncogenes Genes Involved in Cancer Chemoresistance

The death receptor-mediated apoptotic pathway is usually stimulated by binding to the death receptors to their cognitive ligands and leads to activation of caspase cascade in the cell (Cacan, E et al., 2015). Apoptosis, also known as programmed cell death, is typically dysregulated in human cancers. (Bai L et al., 2014) the intrinsic (mitochondrial) and the extrinsic (death receptor) are the two major apoptotic pathways, and they have been extensively studied (Fernald K et al., 2014). Death receptor (DR4) contains a cytoplasmic death domain that activates apoptosis by interacting with tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) through the extrinsic death receptor pathway. The aberrant expression of DR4 has been observed in several human malignancies, including colorectal cancer (van Geelen CM et al., 2006) Furthermore, it has been reported that abnormal expression of DR4 was also correlated with the prognosis of colorectal cancer (van Geelen CM et al., 2006).

Similarly, FAS Apoptosis, a form of programmed cell death, is an essential physiological process responsible for maintaining tissue homeostasis and eliminating damaged or unwanted cells. The regulation of apoptosis is mediated through two primary pathways: the extrinsic pathway and the intrinsic pathway (Peth et al., 2013). The extrinsic pathway, often referred to as the death receptor pathway, is initiated by extracellular signals that activate specific cell surface receptors. Among these, the FAS receptor (Also known as CD95 or Apo-1) plays a critical role in initiating apoptosis.

The extrinsic pathway begins when FAS ligand (FasL), a surface molecule expressed on T lymphocytes and natural killer cells, binds to FAS receptors on the target cell's surface. This interaction forms a death-inducing signaling complex (DISC), which subsequently activates caspase cascades, leading to the execution of apoptosis (Cacan & Ozmen, 2020). This process is vital for immune system regulation, as it allows T cells and natural killer cells to eliminate damaged or infected cells effectively.

FAS belongs to the tumor necrosis factor (TNF) receptor superfamily, which includes receptors such as death receptor (DR4) and death receptor (DR5). These receptors are ligand-activated and transmit apoptosis signals upon binding to their respective ligands, such as the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (Walczak et al., 1997; Wiley et al., 1995). FAS, in particular, is encoded by the FAS gene and is characterized by an intracellular death domain (DD), a critical region for initiating downstream apoptotic signaling (Nagata, 2004). First identified in 1984, FAS is a type-1 transmembrane protein primarily found in a homotrimeric form (Biggin et al., 1984). Its extracellular N-terminus is cysteine-rich and interacts with its ligand (mFasL), while the intracellular DD recruit's adapter proteins such as Fas-associated death domain (FADD) and caspase-8 (Schneider & Tschopp, 2000). This recruitment is a crucial step in activating the caspase cascade and facilitating apoptosis. The FAS-mediated apoptotic pathway has significant implications in immune regulation, cancer biology, and therapeutic development. Dysregulation of FAS signaling can lead to various pathological conditions, including autoimmune diseases and cancer, where cells evade apoptosis and continue to proliferate unchecked. Understanding the molecular mechanisms of FAS and its associated signaling pathways provides valuable insights into therapeutic targets for diseases involving defective apoptosis.

On the other hand, the E2F family of transcription factors plays a pivotal role in regulating genes involved in cell proliferation, differentiation, and apoptosis. These factors can be broadly classified into activators (E2F1 , E2F2, E2F3A) and repressors (E2F3B, E2F4, E2F5, E2F6, E2F7, and E2F8), based on their ability to either activate or inhibit transcription via E2F response elements. However, this classification does not fully capture the pleiotropic nature of E2F activity. E2Fs

contribute not only to cell cycle progression but also regulate apoptosis, differentiation, and DNA damage response, highlighting their complex and context-dependent functions (Endo-Munoz L et al., 2009).

Among these, E2F7 is unique due to several distinctive features. It encodes a protein with two DNA-binding domains but lacks domains necessary for dimerization, transcriptional activation, and binding to the retinoblastoma (Rb) protein. Unlike activator E2Fs, E2F7 acts as a transcriptional repressor, blocking the activation of certain E2F target genes and suppressing cellular proliferation in mouse embryonic fibroblasts (De Bruin A et al., 2003).

Recent studies have shown that miR-595 expression is decreased in non-small cell lung cancer (NSCLC) tissues and cell lines, while E2F7 mRNA is significantly upregulated. Overexpression of miR-595 in A549 cells suppressed E2F7 at both mRNA and protein levels, whereas silencing miR-595 in H358 cells enhanced E2F7 expression. Bioinformatics analyses predicted that E2F7 is a direct downstream target of miR-595, with a potential binding site in the 3′ untranslated region (3′-UTR) of E2F7 mRNA (Bai Q et al., 2020).

Chromatin immunoprecipitation sequencing (ChIP-seq) has revealed that E2F7 preferentially binds genomic sites resembling the E2F consensus sequence. Aberrant expression of E2F7 has been associated with various cancers; for example, its increased expression is linked to cutaneous squamous cell carcinoma, whereas decreased expression is noted in ovarian cancer. However, its role in breast cancer remains unclear (Chu J et al., 2015). Notably, E2F family functions are highly context-dependent, with both tumor-suppressive and oncogenic activities reported depending on tissue type and cellular environment (Saenz-Ponce N et al., 2018).

Finally, Ets homologous factor (EHF) is a key member of the transcription factor network that regulates gene expression in the airway epithelium in response to endogenous and exogenous stimuli (Fossum SL et al., 2017). ETS homologous factor (EHF)plays a critical function in epithelial cell differentiation and proliferation. However, the roles of EHF in cancer remain largely unknown. The function experiments revealed that EHF overexpression promoted CRC cell proliferation, migration, and invasion in vitro and in vivo. the expression levels, precise function and mechanism of

EHF in colorectal carcinoma (CRC). We observed significantly elevated EHF expression in CRC cell lines and tissues. EHF overexpression correlated positively with poor differentiation, advanced T stage, and shorter overall survival of CRC patients (Gao L, et al., 2021). Loss of EHF expression has been revealed in several epithelial carcinomas, including bladder, oral squamous, and breast ductal carcinomas, and a downregulation of EHF expression is associated with prostate cancers .While overexpression of EHF in breast cancers is not associated with poor prognosis of progression-free survival, the expression level of EHF has been revealed to be significantly higher in primary lung cancer tissues compared with adjacent non-tumor tissues of the same patients Data obtained from public datasets indicate that a high level of EHF expression is associated with poor prognosis of patients with NSCLC, and that loss of EHF expression impairs the invasion and the metastasis of lung cancer cells .These studies suggest that EHF may have distinct tissue-specific functions in various cancers . EHF has been revealed to promote lung cancer cell progression but inhibit epithelial carcinomas and prostate cancer growth. The different signaling pathways of EHF in lung cancer cells vs. epithelial carcinomas remain unknown (Wang L et al., 2020).

2.7.1. Investigation of miRNA-595 Regulation of the E2F7 Gene in Cancer Cells

We further want to determine the relationship between the above-mentioned miRNAs and potential target genes. For this purpose, we selected target genes that have been linked to some of these miRNAs based on data analysis (target scan human) and are associated with colorectal cancer. For example, miR-595 target-E2F7.

Previous studies have found that microRNAs can regulate the expression of downstream tumor-related genes and affect the occurrence, development, and metastasis of tumors, including non-small cell lung carcinoma (NSCLC) (Yang Z1 et al., 2017). Research on the function and potential molecular mechanisms of miRNAs in tumors has been a hot topic over the last decade.

Subsequently, we predicted that E2F7 is a potential downstream target of miR-595 via bioinformatics analysis. The potential binding site of miR-595 in the 3'-UTR of E2F7 is shown in Figure (2.5). E2F7 mRNA expression was significantly upregulated in tumor

tissues and cell lines. The mRNA and protein expression levels of E2F7 were significantly inhibited in the miR-595 overexpression group in A549 cells. Conversely, in H358 cells, the expression of E2F7 was enhanced after silencing miR-595 (Bai Q et al., 2020).

Figure 2.5. miR-595 targeting the E2F7 gene via the 3' UTR (Bai Q et al., 2020).

MicroRNAs are pivotal regulators of gene expression and play critical roles in various cancers, including colorectal cancer. The aim of this study was to explore the therapeutic potential of miR-595 in regulating the oncogene E2F7 in the colorectal cancer cell line HT-29. Previous results indicated that E2F7 is upregulated in response to docetaxel treatment in HT-29 cells, contributing to chemoresistance. We hypothesized that miR-595 could downregulate E2F7, thereby enhancing the sensitivity of HT-29 cells to docetaxel.

To test this hypothesis, HT-29 cells were treated with docetaxel, and E2F7 expression levels were measured. We observed a significant upregulation of E2F7 in the treated cells compared to untreated controls. Subsequently, we introduced miR-595 into the HT-29 cells using Lipofectamine-mediated transfection and assessed the effects on E2F7 expression.

2.8. Role of Targeted Therapies in Cancer Treatment

2.8.1. Role of lapatinib in cancer

The human epidermal growth factor receptor (HER) family of receptor tyrosine kinases plays a crucial role in the biology of many cancers. In breast and gastrointestinal cancers, and to a lesser extent in other tumor types, HER2 and its homo- or heterodimerization with HER1 or HER3 are essential for cancer cell growth and survival. Lapatinib, an oral dual tyrosine kinase inhibitor, blocks HER1 and HER2

tyrosine kinase activity by binding to the ATP-binding site of the receptor's intracellular domain, leading to the inhibition of tumor cell growth. In 2007, lapatinib was approved in combination with capecitabine for patients with advanced HER2-positive breast cancer who had progressed following standard therapies with anthracyclines, taxanes, and trastuzumab (Geyer et al., 2006). In 2013, the approval was extended to include a chemotherapy-free combination with trastuzumab for patients with metastatic HER2-positive breast cancer (Gianni et al., 2013). Since 2010, lapatinib has been approved in combination with letrozole for the treatment of postmenopausal women with advanced HER2- and hormone receptor-positive breast cancer (Di Leo et al., 2010). The use of lapatinib in colorectal cancer, particularly in HER2-positive refractory metastatic colorectal cancer, is an emerging area of research. Treatment with lapatinib has shown potential in selected patients, both within its approved indications and in further indications such as HER2-positive refractory metastatic colorectal cancer (Sosnowski et al., 2011; Luo et al., 2014).

Lapatinib ditosylate is an orally administered dual receptor tyrosine kinase inhibitor (TKI) targeting two members of the HER family of receptors: HER1 (EGFR1/ErbB1) and HER2/c-neu (ErbB2). Lapatinib interacts intracellularly by reversibly binding to the cytoplasmic ATP-binding site of the tyrosine kinase domain. This binding prevents phosphorylation and activation of the receptor, thus inhibiting several downstream signaling pathways, including the extracellular signal-regulated kinase 1/2 (ERK1/2) and the phosphatidylinositol 3′-kinase (PI3K)/AKT pathways, which are involved in cell proliferation and apoptosis (Voigtlaender et al., 2018; Bhagyalalitha, M et al., 2012). By blocking these signaling pathways, lapatinib effectively suppresses cancer cell proliferation and induces cell death.

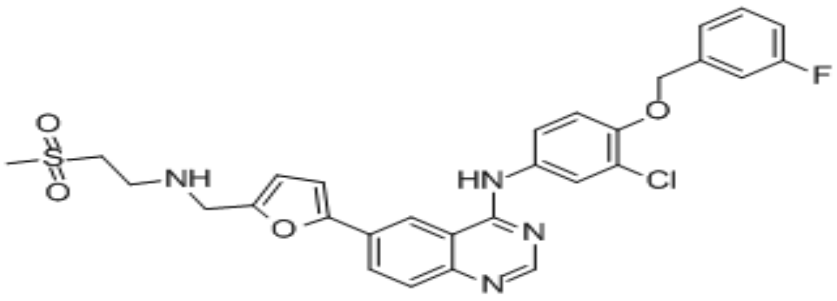

Figure 2.6. Chemical structure of lapatinib.

Lapatinib binds to the tyrosine kinase domain of HER1 and HER2, blocking the ATP-binding site and preventing activation of downstream signaling cascades. This inhibition is symbolized by "X" in the diagram, which illustrates the blockade of pathways such as JNK, MAPK, MEKK, and PI3K, all of which are critical for cancer cell proliferation and survival.

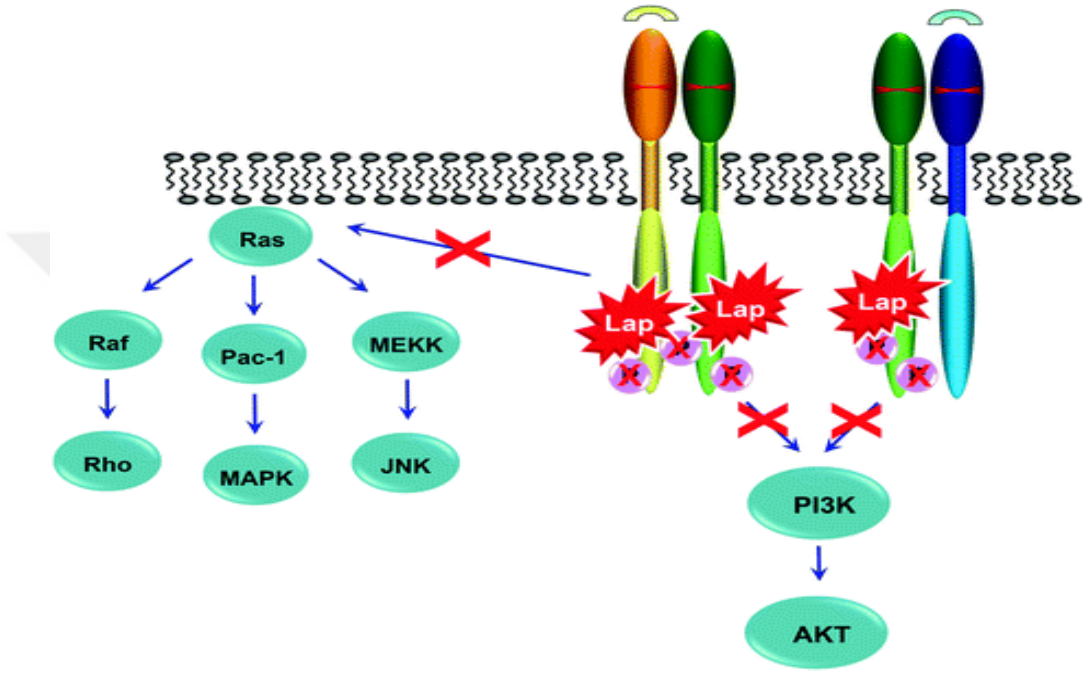

Figure 2.7 Intracellular action of lapatinib (Voigtlaender M et al., 2018).

2.8.2. Role of docetaxel in cancer

Docetaxel (Doc), a semisynthetic analog of paclitaxel, it has been used for the treatment of various human malignancies (Fan R et al., 2011). Docetaxel, which belongs to the taxane class of anticancer agents, has a broad spectrum of pro-apoptotic and antitumorigenic activities against various human cancers from prostate, breast, lung, ovarian, prostate, head and neck. Docetaxel disrupts microtubules by altering tubulin polymerization and depolymerization. Disturbance in microtubule assembly by docetaxel leads to induction of p53 tumor suppressor and p21 cell cycle inhibitor and thus to cell cycle arrest at G2-M phase. Docetaxel has been reported not only to inhibit growth, but also to induce apoptosis in various cancer cells (Kim IY et al., 2011).

Docetaxel is a chemotherapeutic has activity against Colorectal cancer (CRC). However, combination therapy with other therapeutic agents is a potential strategy to enhance the efficacy of docetaxel in CRC treatment. The nuclear factor-κB (NF-κB) signaling pathway is implicated in a variety of malignancies (e.g., CRC), and the blockade of NF-κB may increase the sensitivity of cancer cells to chemotherapy (Zou Y et al., 2021).

3. MATERIAL AND METHODS

3.1. Material

1. Cell Lines: HT-29, SW-620, HCT-116
2. Culture Medium: McCoy media or DMEM, supplemented with 10% FBS, 1% penicillin-streptomycin , and any additional supplements as needed All culture media and supplements were purchased from Capricorn Scientific, Germany.
3. Trypsin-EDTA: For cell detachment. From (Biological Industries)
4. Phosphate-Buffered Saline (PBS): For washing cells.

3.1.1. Chemotherapeutic drugs

1. Docetaxel (LC Laboratories)
2. Lapatinib (LC Laboratories)

3.1.2. Cell analysis equipment

1. Biological Safety Cabinet
2. CO_2 Incubator
3. Centrifuge
4. Inverted Microscope

3.1.3. Additional laboratory supplies:

1. Pipettes and Pipette Tips
2. Sterile Bottles and Tubes
3. Vortex Mixer: For mixing samples
4. Water Bath: For warming reagents and media.

3.2. RT-PCR Materials:

1. RNA Extraction Kit QIAGEN, Hilden, Germany
2. cDNA Synthesis Kit Applied Biological Materials Inc.
3. RT-PCR Master Mix Applied Biological Materials Inc.
4. Primers
5. PCR Plates or Tubes

3.3.Gene and MiRNA Targeting:

1. miRNA Agomirs or Antagomirs Applied Biological Materials Inc.
2. Transfection Reagents: Lipofectamine Applied Biological Materials Inc.

3.4.Safety and Cleanliness:

1. 70% Ethanol: For disinfecting surfaces and equipment.
2. Bleach: For cleaning up spills and decontaminating surfaces.
3. Personal Protective Equipment (PPE): Lab coats, gloves, safety glasses.

3.5.Data Analysis:

Software: For analyzing RT-PCR data, such as the software provided with your PCR machine or other statistical analysis tools.

3.6. Methods

3.6.1. Cell culture

The three human colorectal cancer cell lines, HCT-116,1HT-29, and SW-620, were used in this study. All cell types were grown in their manufacturer-approved media—HCT-116 in McCoy's 5A, SW-620 in Eagle's Minimum Essential Medium (EMEM), and HT-29 in Dulbecco's Modified Eagle's Medium (DMEM). Media was also supplemented with 10% FBS and 1% Penicillin-Streptomycin. The cells were cultured at 37°C in a 5% CO_2 atmosphere in a humidified incubator until they reached about 80% confluence. Cell viability was determined by using 0.4% trypan blue staining (Biological Industries).

The UV light was turned on for about 30 to 60 minutes before and after each cell culture session in the cell culture room and hood. The level of contamination was monitored regularly. All cell culture experiments were conducted in an ESCO class II type A2 cell culture hood. The surfaces in the cell culture hood were cleaned with 70% ethanol and 10% bleach.

HCT-116 frozen cells were thawed in McCoy's 5A medium and HT-29 frozen cells were thawed in 10 mL DMEM medium and SW620 frozen cells were thawed in (Eagle'sMinimum Essential Medium) and transferred into 50mL canonical tubes using Pasteur pipettes, then centrifuged at 3000 rpm for 3 min to remove the DMSO present in the freezing media and its effect. After discarding the supernatant, the cell pellets were added to 10 mL media, and the cells were distributed into the T 75 cell culture flask. For further passages, after washing the flask with 10 mL of PBS, confluent cells were detached by adding 5 mL of prewarmed trypsin for cell culture flasks. The flasks were incubated at 37 ºC for approximately 3-5 minutes until the cells were completely detached from the surface of the flasks and then 10 ml of pre-warmed media were added to the flask. After pipetting with serological pipettes, the media were transferred to 50-mL canonical tubes and centrifuged at 3000 rpm for 3 minutes. After discarding the supernatant, the cell pellets were dissolved in 10 mL of media, and the cells were seeded onto the plates at the desired dilution, taking cell density into account. The total volume of media in a T75 24 flask was made up to 20 mL. The confluence of the cells was continuously checked to avoid over-confluence.

3.6.2. Cell viability screening (MTT assay)

The viability of cancer cells under the influence of drugs was measured using the MTT ((3-(4, 5-dimethyl thiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay. Normally active cells metabolize MTT yellow tetrazolium and the color changes to purple formazan due to the action of dehydrogenase NADPH enzymes.

For the MTT assay, 96-well plates were used in which 7500 cells per well were seeded in a 200µl medium. 24 hours after seeding the cells, treatment with the drug (lapatinib and docetaxel) was performed in 4 replicates for each concentration and the cells were incubated for 48 hours. MTT powder was dissolved in phosphate buffered saline (PBS) and filtered using filters with a pore size of 0.2 microns in a cell culture hood to make the MTT solution (5 mg/ mL). The MTT solution was kept frozen at -20 C in the dark. The MTT solvent was made by diluting MTT in 90% serum-free EME. In the MTT assay, the medium was carefully aspirated from the wells and each well was washed with 100µl PBS. Then, 10% diluted MTT in 90% serum-free EME was added to each well. (The final MTT concentration in the wells was 0.5 mg/mL) and the plates were incubated in a humidified 5% CO2 incubator at 37 ºC for 4 hours. After incubation, we remove the MTT solution from each well. Then, 150µL of DMSO was added to each well and the plates were incubated in the dark on an orbital shaker for 1 hour.

Spectrophotometric measurement was performed using a Multiskan Go microplate reader (ThermoScientific) at two different wavelengths of 540 and 570 nm and absorbance values were used for 25 data analyses. The spectrophotometric signal of formazan correlates with the number of cells.

Table 3.1. MTT viability assay.

Material	Volume	Incubation	Purpose
7500 cells	200 µl	24 hours	Viability
docetaxel	0.05,0.1,0.5, and 10uM	48 hours	Treat cell
Lapatinib	0.1,0.5,1,5, and 10uM	48 hours	Treat cell
PBS	100 µl	Non	Washing cells and removing dead cells
MTT dye	100 µl	4 hours	Change color notification, and, dehydrogenase NADPH enzymes by mitochondria
DMSO	150 µl	30 min.	Remove MTT, and replace by 150 µl DMSO

3.6.3. Cell migration and wound healing (Scratch Assay)

The migration of cells and the healing of wounds are both convincing indicators of cell vitality. Many pathological processes, such as inflammation and cancer, can be triggered and involve cell migration. We seeded 800000 cells per well in 6-well plates (Costar, Corning, flat bottom), scratched each well with a 200µl-pipet tube tip size to create a scratch line, divide the circle of each well into two equal radii, and then incubated the cells for 24 hours. The cells in each well were treated with different concentrations of docetaxel and lapatinib the next day, and the cells were then incubated for another three days to observe cell migration towards the scratch line.

3.6.4. RNA extraction

500.000 cells were seeded per well in 6-well plates (Costar, Corning, flat bottom) and the cells were incubated for 24 hours. The ic50 value concentrations of docetaxel and lapatinib were administered and the cells were further incubated for 24 hours. The media were removed from each well and the wells were washed with cold PBS. After washing the cells, cell harvesting was started by adding 1 ml (1000µl) of QIAzol lysis reagent (Qiagen, Germany) to maintain the integrity of RNA due to the highly effective inhibition of RNase activity during cell breakage and cracking and dissolution of cell components in the process of sample homogenization. Into each well and the plates were stored at room temperature for 5 min (with pipetting and shaking during the incubation period to detach all cells from the surface of the plate). The cells were then transferred to a 1.5 ml Eppendorf tube and 200µL chloroform was added to each tube to separate the aqueous phase containing RNA from the organic phase containing other organic particles of the cells and the tubes were shaken for one minute. After the tubes were placed in the tube rack for 3 minutes, centrifugation was performed at 10,500 rpm for 10 minutes at 4°C. Then, the top layer containing RNA molecules was carefully transferred to a new 1.5-ml tube, avoiding other layers containing other cell particles and 500 µl isopropyl alcohol was added for each tube, briefly shaking the tubes up and down by hand and then incubated for 10 minutes at room temperature. Due to the presence of NH 4+, the addition of isopropyl alcohol keeps the free nucleotides in solution, separating them from the precipitated RNA, After the incubation period, the tubes were centrifuged at 10,500 rpm for 10 minutes at 4°C. The supernatant was then aspirated and discarded with care. Each tube received 1 ml of 75% absolute ethanol dissolved in 25 percent RNA-free water, and the tubes were centrifuged at 7500 rpm for 5 minutes at 4 C. The RNA pellet in the tubes was air dried to remove all ethanol residues after the supernatant was completely removed (the tubes were placed on a tube rack in a sterile hood environment). Finally, the RNA pellets were redissolved in 40 µl of RNase-free water, and 1 µl was used to measure the RNA concentration using a spectrophotometer at two wavelengths (260 nm and 280 nm) in duplicate. Concentrations were calculated by taking the average of each replicate in specific absorbance minus the average of RNAase replication at 280nm. The average of the 260nm replicates was divided by the average of the 280nm replicates to determine the RNA purity ratio. Isolated RNA samples were stored at -82 ºC in the freezer before being

used in qRTPCR experiments. Extensive freeze-thaw cycles were avoided so as not to compromise RNA quality. RNA isolation experiments were performed in a fume hood to avoid inhalation of evaporating chemicals.

3.6.5. Quantitative real-time polymerase chain reaction (qRT-PCR)

LightCycler capillaries (20 µL, Roche) were used with the Roche Light Cycler 1.5 instrument (Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany). For the reverse transcription reaction in the RT-PCR process, each 20 µL reaction mixture consisted of qRT-PCR Master Mix (10 µL, 1x), forward primer (1 µL), reverse primer (1 µL), template RNA (50 ng per reaction), and RNase-free water added to reach the final volume. Reactions were run in duplicates, and real-time RT-PCR was performed using the thermal cycling protocol, which included the following steps: one cycle of reverse transcription at 50 °C for 25 minutes, one cycle of initial denaturation at 95 °C for 15 minutes, followed by 40 cycles of three-step amplification denaturation at 95 °C for 15 seconds, annealing at 60 °C for 30 seconds (with fluorescence detection), and extension at 72 °C for 35 seconds. Finally, the reactions underwent a cooling step at 37 °C for 30 seconds.

Table 3.2. Volumes of each reaction used in qRT-PCR experiments.

Material	Volume for one reaction	Concentration
Extracted RNA	4µl	20ng/µl
qRT-PCR Master Mix	10 µl	1X
Reverse primer	1µl	µM
Forward primer	1µl	µM
RNase water	4µl	100% free

Table 3.3. Reaction using in light cycler qRT-PCR.

Analysis mode	Number of cycles	Analysis mode	Temperature (^{0}C)	Duration (minute)	Acquisition mode
Rev. Transcription	1	None	50	25	None
Denaturation	1	None	95	15	None
Amplification	40	Quantification	95	15	None
			60	30	None
			72	35	Single
Cooling	1	None	37	30	None

Relative RNA quantification was performed using the following formula:

R = 2-{Cp(sample) - Cp(control)} .

For normalization, GAPDH primers were used in all RT-PCR experiments. In each RT-PCR run, each sample was analyzed in duplicate (two technical replicates) for every condition, along with duplicate reactions using GAPDH primers under the same conditions. All primers were purchased from Macrogen and dissolved in nuclease-free water by gentle pipetting, without vortexing, and stored at -20 C. Primers were used in RT-PCR experiments as follows:

Table 3.4. Primers were used in the quantitative real-time PCR experiments.

miRNA	Forward And Reverse Primers
MIR-595	Forward : GAAGTGTGCCGTGGTG Reverse : GGTCCAGTTTTTTTTTTTTTTAGAC
miR-718	Forward : CTTCCGCCCCGC Reverse : GTCCAGTTTTTTTTTTTTTTCGAC
miR-206	forward : GCAGTGGAATGTAAGGAAGT Reverse : CCAGTTTTTTTTTTTTTTCCACACA
miR-1199	Forward : CCGGGCCGCG Reverse : GGTCCAGTTTTTTTTTTTTTTCTG

Genes	Forward and Reverse Primers
FAS	Forward : 50-GCG ATG AAG AGC ATG GTTTAG-3′, Reverse : 50-GGC TCA AGG GTTCCA TGT T-3′,
DR4	Forward : 5’- CTGAGCAACGCAGACTCGCTGTC -3’ Reverse : 5’- TCAAAGGACACGGCAGAGCCTGTG -3’
E2F7	Forward : 5’-GGAAAGGCAACAGCAAACTCT -3’ Reverse : 5’-TGGGAGAGCACCAAGAGTAGAAGA -3’
EHF	Reverse : 5'-GAAGAACAACAGCAGCATGACC-3' Reverse : 5'-TCAGGTTTCGGTGTATGAGTTG-3'
GAPDH	Forward : 5′-AAGGTGAAGGTCGGAGTCAA-3′ Reverse : 5′-AATGAAGGGGTCATTGATGG-3′

3.6.6. Overexpression of miR-595

Suspension cells (200,000 per well) were seeded in 2 mL of serum-free, antibiotic-free medium in 6-well plates and incubated at 37°C in a 5% CO_2 incubator until 70–90% confluency. For transfection, miRNA was diluted to 50 nM in 100 µL medium (Solution A), and 10 µL of RNAifectin™ was diluted in another 100 µL (Solution B). Both solutions were incubated separately at room temperature for 5 minutes, then combined and incubated for 20 minutes to form RNA/oligo-liposome complexes.

A total of 200 µL of the transfection complex was added to 800 µL of serum-free medium containing the cells. Plates were incubated for 5–8 hours at 37°C. Following incubation, 2 mL of complete growth medium with serum was added directly to each well without removing the transfection mixture. Cells were maintained at 37°C, and gene exression was monitored between 6- and 72-hours post-transfection.

3.6.7. Data analysis and visualization

All experiments were performed in triplicate. Data are presented as mean ± standard deviation (SD). Statistical comparisons between treated and control groups were performed using Student's t-test. Significance was indicated as follows: *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ***P ≤ 0.0001. GraphPad Prism software was used to perform statistical analyses and visualize the MTT and qRT-PCR results.

4. RESULTS

4.1. Docetaxel and Lapatinib Decreased Viability of Colorectal Cancer Cell Lines in a Dose-Dependent Manner

Impact of docetaxel and lapatinib on HT-29 cell viability

The results showed that HT-29 cell viability decreased with increasing concentrations of docetaxel, particularly between 0.05 µM and 1 µM, while no significant difference was observed at 10 µM. Similarly, when cells were treated with lapatinib at concentrations of 0.1, 0.5, 1, 5, and 10 µM, cell viability remained unaffected at 0.1, 0.5, and 1 µM. However, a marked decrease in viability was observed at 5 µM and 10 µM, with reductions ranging from 40% to 60%. A slight sensitivity was noted at 1 µM, but it was not substantial.

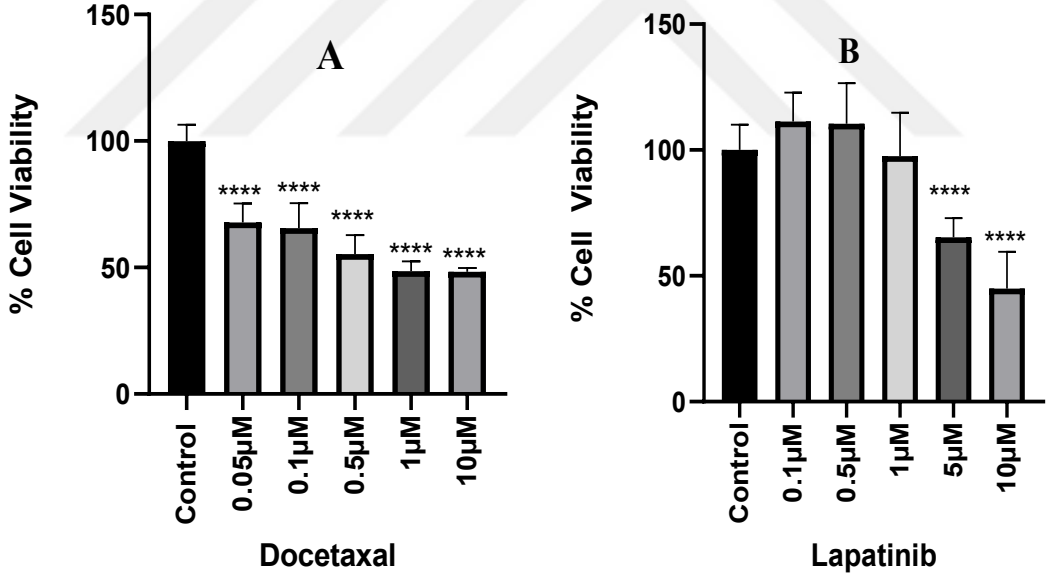

Figure 4.1. HT-29 colorectal cancer cells were treated with different concentrations of (A) docetaxel and (B) lapatinib for 48 hours. Cell viability was assessed using the MTT assay, and results are expressed as the percentage of viable cells relative to untreated controls. Experiment was repeated three times with consistent results. Statistical analysis was performed using a t-test against control groups (****: $P \leq 0.0001$; ***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$).

Impact of docetaxel and lapatinib on HCT-116 cell viability.

While the results showed that HCT116 cell lines were treated with different doses of docetaxel (0.05, 0.1, 0.5, 1, 10 µM), high doses of docetaxel showed A significant decrease in viability was observed after inoculation. Whereas for lapatinib viability decreased with increasing concentrations of lapatinib, which was particularly evident at 5 µM and 10 µM. 0.1, 0.5, 1,5 and 1 µM of lapatinib treatment killed about 20%. The cell viability decreased by 35-40% at 5 µM and 20-25% at 10 µM.

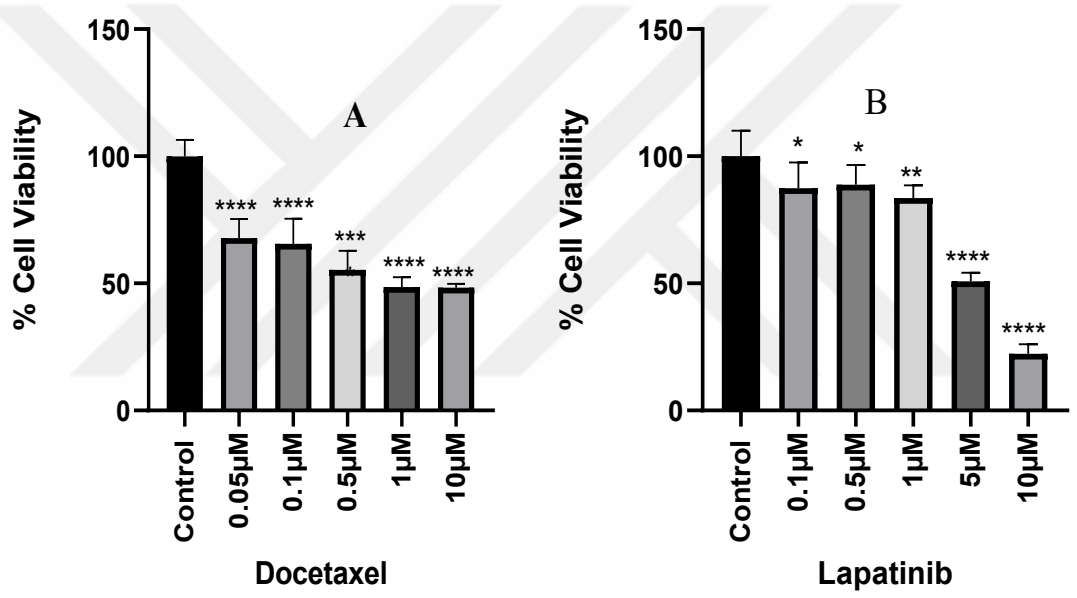

Figure 4.2. HCT-116 colorectal cancer cells were treated with different concentrations of (A) docetaxel and (B) lapatinib. Cell viability was assessed using the MTT assay, and results are expressed as the percentage of viable cells relative to untreated controls. The experiment was repeated three times with consistent results. Statistical analysis was performed using a t-test against control groups (****: $P \leq 0.0001$; ***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$).

Impact of docetaxel and lapatinib on SW-620 cell viability

While the results show SW-620 colorectal cancer cells were treated with different concentrations of docetaxel (0.05, 0.1, 0.5, 1, and 10 µM). A significant decrease in cell viability was observed at higher doses of docetaxel after 24 and 48 hours of treatment. For lapatinib, the cells were treated with 0.1 µM, 0.5 µM, 1 µM, 5 µM, and 10 µM concentrations. The results showed that cells remained viable at 0.1 µM, 0.5 µM, and 1 µM, while cell viability decreased significantly with increasing concentrations of lapatinib. We concluded that 0.1 µM, 0.5 µM, and 1 µM of lapatinib did not cause toxicity in SW-620 cells, whereas higher concentrations (5 µM and 10 µM) significantly reduced cell viability.

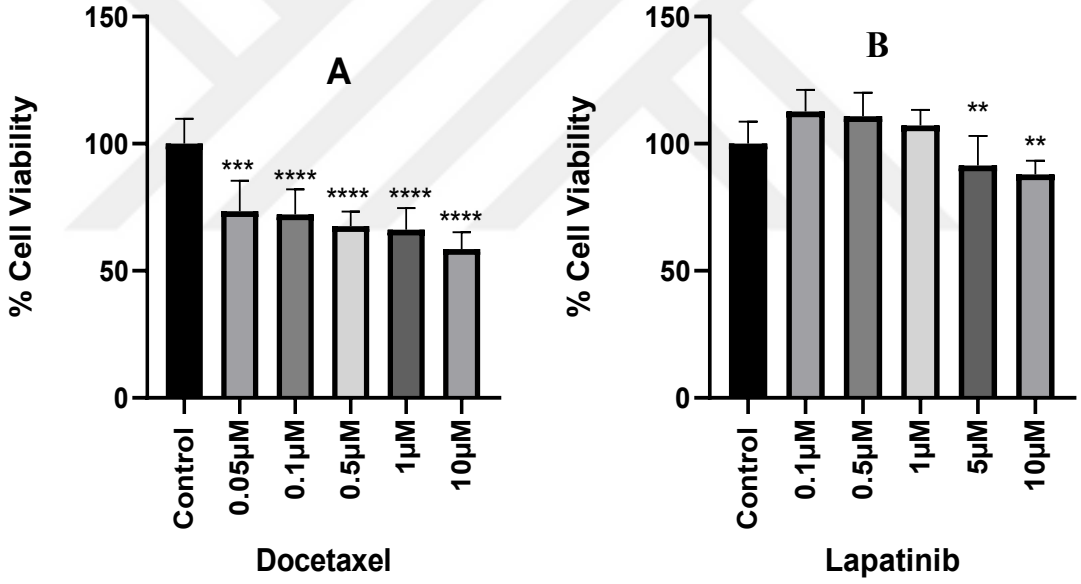

Figure 4.3. SW-620 colorectal cancer cells were treated with different concentrations of docetaxel (0.05, 0.1, 0.5, 1, and 10 µM) and lapatinib (0.1, 0.5, 1, 5, and 10 µM) for 48h Cell viability was determined were analyzed by the MTT assay, and the results are expressed as 100 viable cells per unit compared to untreated controls. The experiment was repeated three times, with consistent results. Statistical analysis was performed using a t-test against control groups (****: $P \leq 0.0001$; ***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$).

4.2.Impact of Docetaxel Treatment on Migration in Chemo-Resistant Colorectal Cancer Cell Lines

Impact of Docetaxel Treatment on HCT-116 Colorectal Cancer Cell Lines

We analyzed microscopic images of HCT-116 colorectal cancer cells were analyzed to evaluate the effects of docetaxel on cell migration over 72 hours. Images were captured at 24, 48, and 72 hours for Control, low (0.05 μM), IC_{50} (0.03 μM), and high (10 μM) docetaxel concentrations.

At 24 hours, cells in all groups exhibited migration toward the scratch line, indicating that cells remained viable under all experimental conditions. By 48 hours, Control cells showed extensive migration, partially closing the scratch gap, whereas cells treated with 0.05 μM and IC_{50} concentrations displayed reduced migration. The highest concentration (10 μM) induced pronounced cell death and morphological alterations. At 72 hours, the scratch gap in the Control group was closed due to active migration. In treated groups, all concentrations caused varying levels of cell death and detachment, with effects most severe at 10 μM, resulting in markedly reduced migration and lower cell viability.

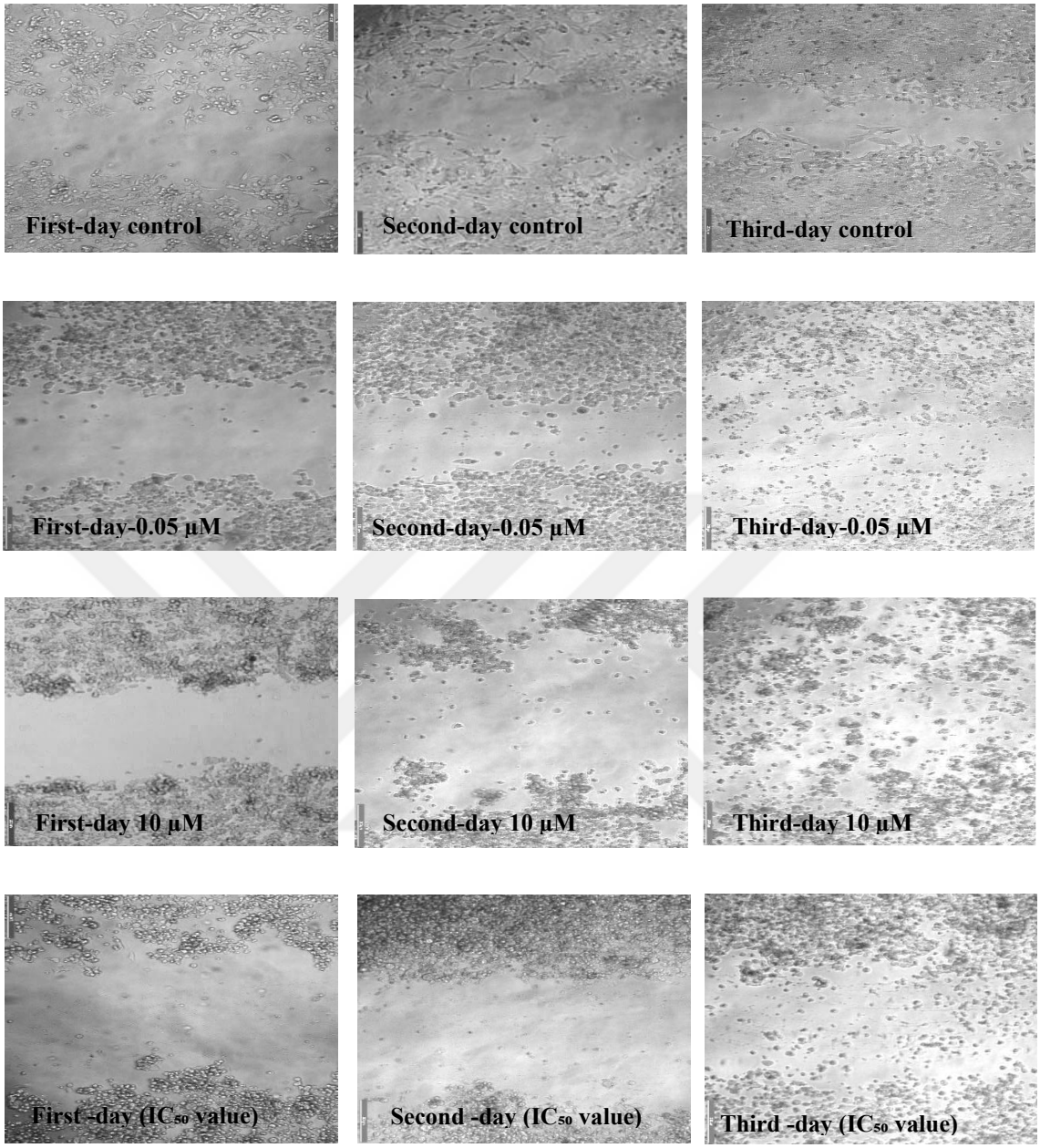

Figure 4.4. Time-dependent effects of docetaxel on HCT-116 colorectal cancer cell migration and morphology. Microscopic images of HCT-116 colorectal cancer cells were captured at 24, 48, and 72 hours after treatment with varying concentrations of docetaxel (Control, 0.05 µM, IC_{50} [0.03 µM], and 10 µM). Images were taken at 10× magnification with a 200 µm scale bar. Cell migration decreased and morphological changes increased with higher docetaxel concentrations over time, with the most pronounced effects observed at 10 µM.

Impact of Docetaxel Treatment on HT-29 Colorectal Cancer Cell Lines

We evaluated microscopic images from cell migration studies on the HT-29 colorectal cancer cell line following treatment with varying concentrations of docetaxel. At 24 hours post-treatment, cells in all groups, including Control, 0.05 µM, 0.03 µM (IC_{50}), and 10 µM, exhibited migration toward the scratch line, indicating that the cells remained viable under all experimental conditions.

By 48 hours, noticeable cell movement was observed only in the Control group, with partial closure of the scratch gap. In contrast, migration was reduced in cells treated with 0.05 µM and IC_{50} 0.03 µM concentrations of docetaxel compared to the Control group, while the highest concentration (10 µM) induced pronounced cell death and morphological alterations.

At 72 hours, the scratch gap in the Control group was completely closed due to active cell migration. Treated groups continued to show reduced migration, with the most severe effects seen at 10 µM, where extensive morphological changes and cell death were evident. These observations collectively demonstrate the dose- and time-dependent inhibitory effects of docetaxel on HT-29 cell migration and viability.

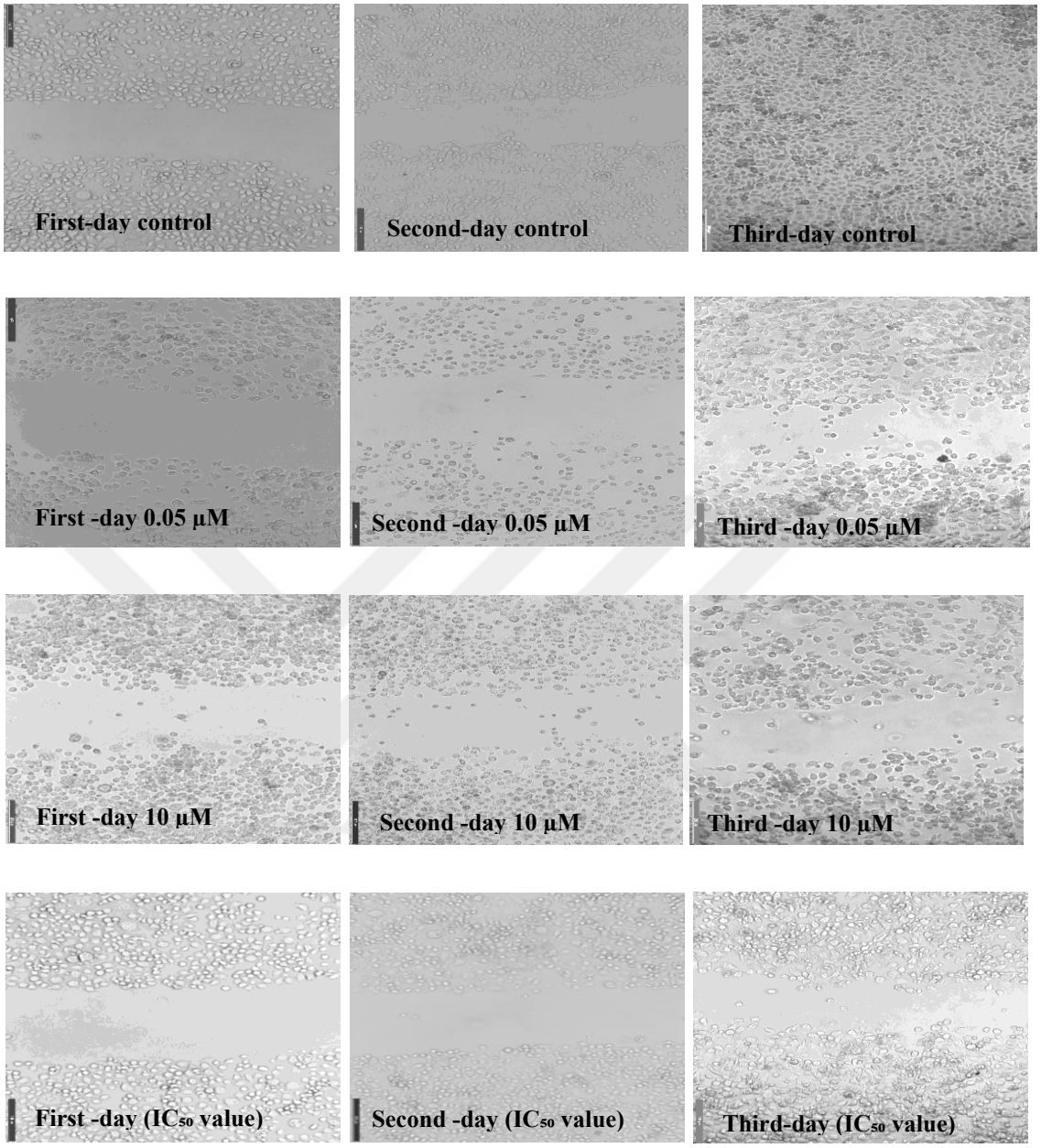

Figure 4.5. Time-dependent effects of docetaxel on HT-29 colorectal cancer cell migration and morphology. Microscopic images of HT-29 colorectal cancer cells were captured at 24, 48, and 72 hours after treatment with varying concentrations of docetaxel (Control, 0.05 µM, IC_{50} [0.03 µM], and 10 µM). Images were taken at 10× magnification with a 200 µm scale bar. Cell migration progressively decreased and morphological changes became more pronounced with higher docetaxel concentrations over time, with the most significant effects observed at 10 µM.

4.2.1. Impact of Lapatinib Treatment on Migration in Chemoresistant Colorectal Cancer Cell Line

Impact of lapatinib Treatment on HCT-116 Colorectal Cancer Cell Lines

We evaluated microscopic images from cell migration studies performed on the HCT-116 colorectal cancer cell line at 24 hours after treatment with specific concentrations of lapatinib. Cell migration toward the scratch line was observed in the Control, 0.1 µM, 5.6 µM (IC_{50}), and 10 µM lapatinib-treated groups, indicating cell viability across the different treatment conditions.

At 48 hours following treatment, noticeable cell movement was observed in the Control group, with partial closure of the migration gap. Cell migration was observed in the groups treated with 0.1 µM and 5.6 µM (IC_{50}) lapatinib. At the high concentration of 10 µM, migration was strongly inhibited, and cells exhibited visible morphological changes and increased cell death.

At 72 h after lapatinib treatment, dose-dependent alterations in cell migration occurred. At 0.1µM cells were viable and migration was enhanced compared to control. A significant increase of migration was achieved at 5.6µM (IC_{50}) and viability of cells was retained. In contrast, at 10µM the migration was significantly suppressed and a decrease in both cell movement, morphological alterations and most importantly reduction of viable cells occurred.

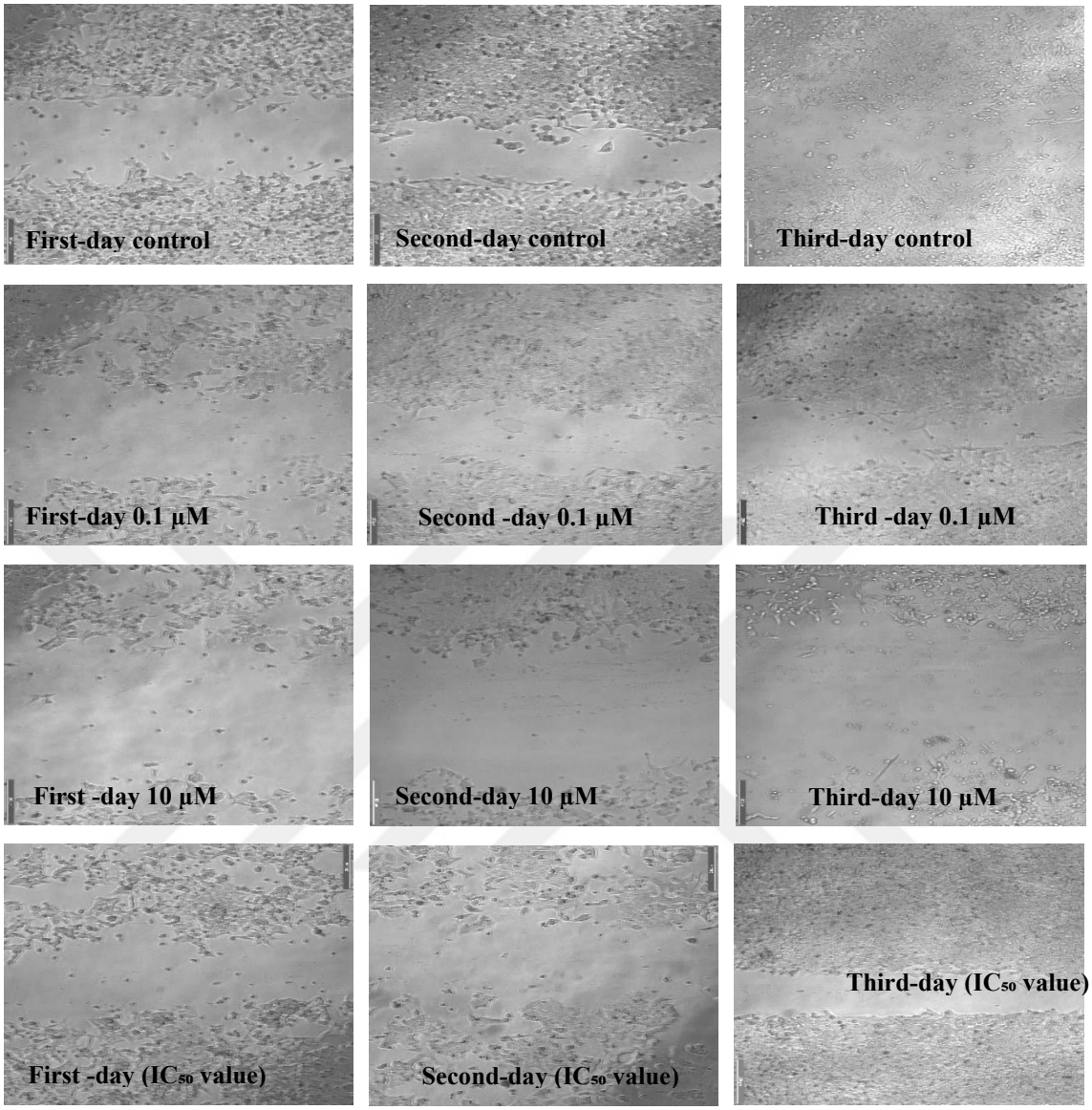

Figure 4.6 Time-dependent effects of lapatinib on HCT-116 colorectal cancer cell migration and morphology. Microscopic images of HCT-116 colorectal cancer cells were captured at 24, 48, and 72 hours after treatment with varying concentrations of lapatinib (Control, 0.1 µM, IC_{50} [5.6 µM], and 10 µM). Images were taken at 10× magnification with a 200 µm scale bar. Cell migration progressively decreased and morphological changes became more pronounced with higher lapatinib concentrations over time, with the most significant effects observed at 10 µM.

Impact of lapatinib Treatment on HT-29 Colorectal Cancer Cell Lines

We evaluated microscopic images from cell migration studies on the HT-29 colorectal cancer cell line following treatment with different concentrations of lapatinib. At 24 hours post-treatment, all groups, including Control, 0.1 μM, 3.9 μM (IC_{50}), and 10 μM lapatinib, exhibited migration toward the scratch line, indicating that cells remained viable under all experimental conditions.

By 48 hours, noticeable cell movement was observed in the Control group, with partial closure of the scratch gap, as well as in cells treated with 0.1 μM and IC_{50} (3.9 μM) lapatinib, though migration was reduced compared to the Control. At the highest concentration (10 μM), there was a pronounced increase in cell death and morphological alterations.

At 72 hours, cell migration continued in the Control, 0.1 μM, and IC_{50}-treated groups, indicating maintained cell viability, whereas cells treated with 10 μM lapatinib showed extensive cell death and morphological changes. These observations demonstrate the dose- and time-dependent inhibitory effects of lapatinib on HT-29 cell migration and viability.

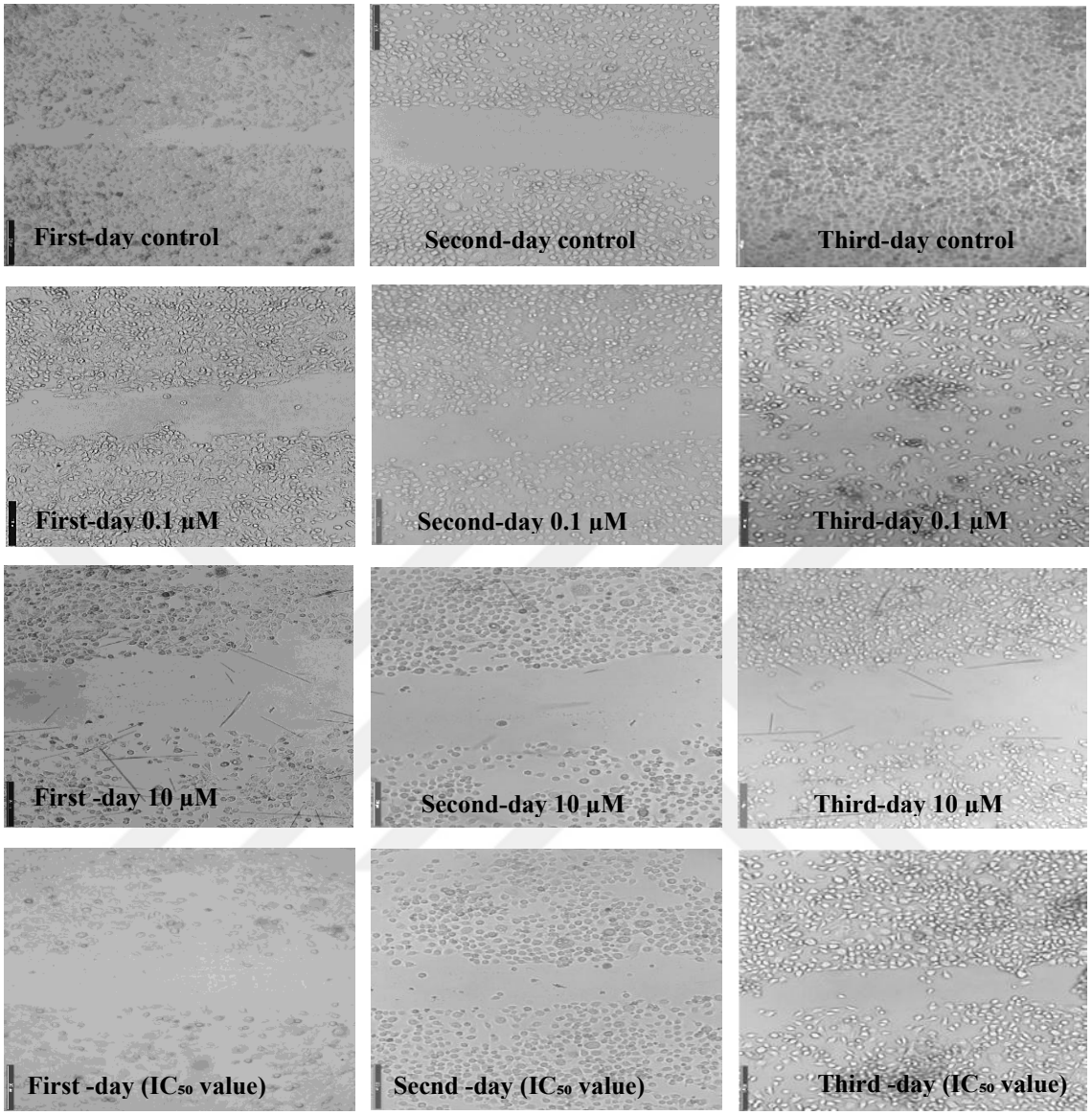

Figure 4.7 Time-dependent effects of lapatinib on HT-29 colorectal cancer cell migration and morphology. Microscopic images of HT-29 colorectal cancer cells were captured at 24, 48, and 72 hours after treatment with varying concentrations of lapatinib (Control, 0.1 µM, IC_{50} [3.9 µM], and 10 µM). Images were taken at 10× magnification with a 200 µm scale bar. Cell migration progressively decreased and morphological changes became more pronounced with higher lapatinib concentrations over time, with the most significant effects observed at 10 µM.

4.3. Effect of Docetaxel and Lapatinib on the Expression of Target MiRNA in Colorectal Cancer Cell Lines.

Effect of docetaxel and lapatinib on miR-595 Expression in HCT-116 and HT-29 colorectal Cancer Cell Lines

The expression of miR-595 was measured after cells were treated with the IC50 values of docetaxel and lapatinib for 24 hours and compared to the control group (no treatment) for both types of cells, HCT-116 and HT-29. Docetaxel increases the expression of MIR-595 transcripts in the colorectal cancer HT-29 cell line by approximately 70% while decreasing it by approximately 50% in the HCT-116 cell line. While using of lapatinib the expression of MIR-595 transcripts in the colorectal cancer HT-29 cell line decreased by approximately 70% while decreasing it by approximately 95% in the HCT-116 cell line.

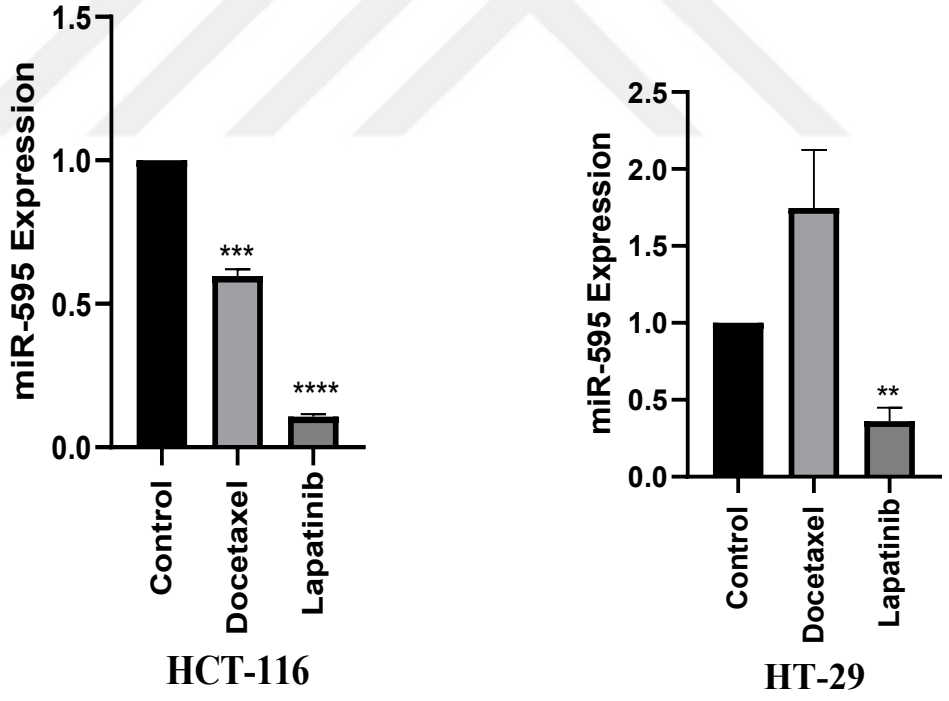

Figure 4.8 Levels of miR-595 transcript1expression in tumor cells (HT-29 and HCT-116). After treatment with docetaxel and lapatinib, cells were harvested, RNA was extracted, and cDNA was synthesized. MiR-595 transcript-specific primers were used in qRT-PCR to generate the data. The obtained data were normalized to GAPDH levels. The results shown in the graphs represent the average of three independent experiments. Statistical

analysis was performed using a t-test against control groups (****: P ≤ 0.0001; ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05).

Effect of docetaxel and lapatinib on miR-718 Expression in HCT-116 and HT-29 colorectal Cancer Cell Lines.

The expression of MIR-718 was measured after cells were treated with the IC50 values of docetaxel and lapatinib for 24 hours and compared to the control group (no treatment) for both types of cells, HCT-116 and HT-29.Docetaxel increases the expression of MIR-718 transcripts in the colorectal cancer HT-29 cell line by approximately 100% while decreasing it by approximately 90% in the HCT-116 cell line. While using of lapatinib the expression of MIR-718 transcripts in the colorectal cancer HT-29 cell line by approximately 40% while decreasing it by approximately 80% in the HCT-116 cell line.

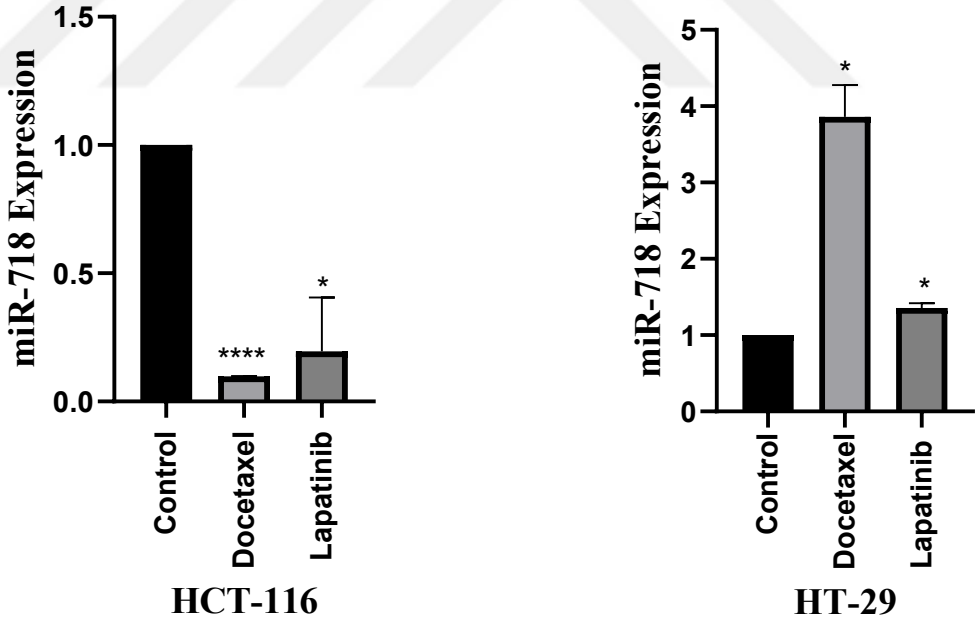

Figure 4.9. Levels of miR-718 transcript expression in tumor cells (HT-29 and HCT-116).

After treatment with docetaxel and lapatinib, cells were harvested, RNA was extracted, and cDNA was synthesized. MiR-59 transcript-specific primers were used in qRT-PCR to generate the data. The obtained data were normalized to GAPDH levels. The results shown in the graphs represent the average of three independent experiments. Statistical

analysis was performed using a t-test against control groups (****: P ≤ 0.0001; ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05).

Effect of docetaxel and lapatinib on miR-206 Expression in HCT-116 and HT-29 colorectal Cancer Cell Lines.

The expression of miR-206 was measured after cells were treated with the IC50 values of docetaxel and lapatinib for 24 hours and compared to the control group (no treatment) for both types of cells, HCT-116 and HT-29. Docetaxel increases the expression of miR-206 transcripts in the colorectal cancer HT-29 and HCT-116 cell line by approximately 50% and 30 %. While using of lapatinib the expression of miR-206 transcripts in the colorectal cancer decrease in both cell line HT-29 and HCT-116 by approximately 80%.

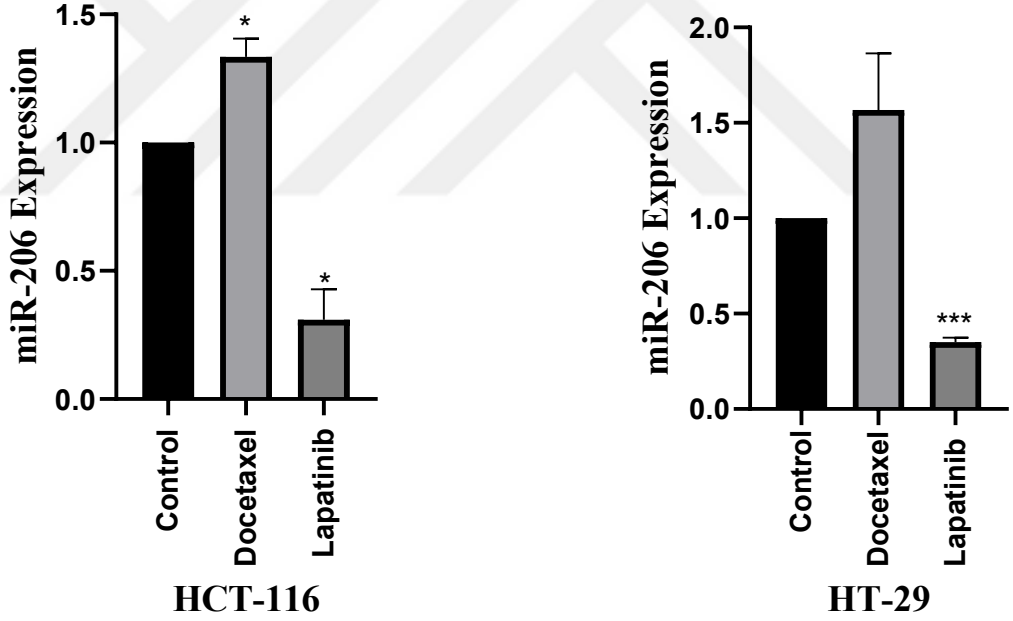

Figure 4.10. Levels of miR-206 transcript expression in tumor cells (HT-29 and HCT-116).

After treatment with docetaxel and lapatinib, cells were extracted, RNA was separated, and cDNA was produced. miR-206 transcript-specific primers were used in qRT-PCR to generate the data. The acquired data were GAPDH level normalized. Statistical analysis was performed using a t-test against control groups (****: P ≤ 0.0001; ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05).

Effect of docetaxel and lapatinib on miR-185 Expression in HCT-116 and HT-29 colorectal Cancer Cell Lines.

The expression of miR-185 was measured after cells were treated with the IC50 values of docetaxel and lapatinib for 24 hours and compared to the control group (no treatment) for both types of cells, HCT-116 and HT-29. Docetaxel decreases the expression of miR-185 transcripts in the colorectal cancer HT-29 and HCT-116 cell lines by approximately 80%. While using lapatinib, the expression of miR-185 transcripts in the colorectal cancer HT-29 cell line decreased by approximately 40% while decreasing by approximately 90% in the HCT-116 cell line.

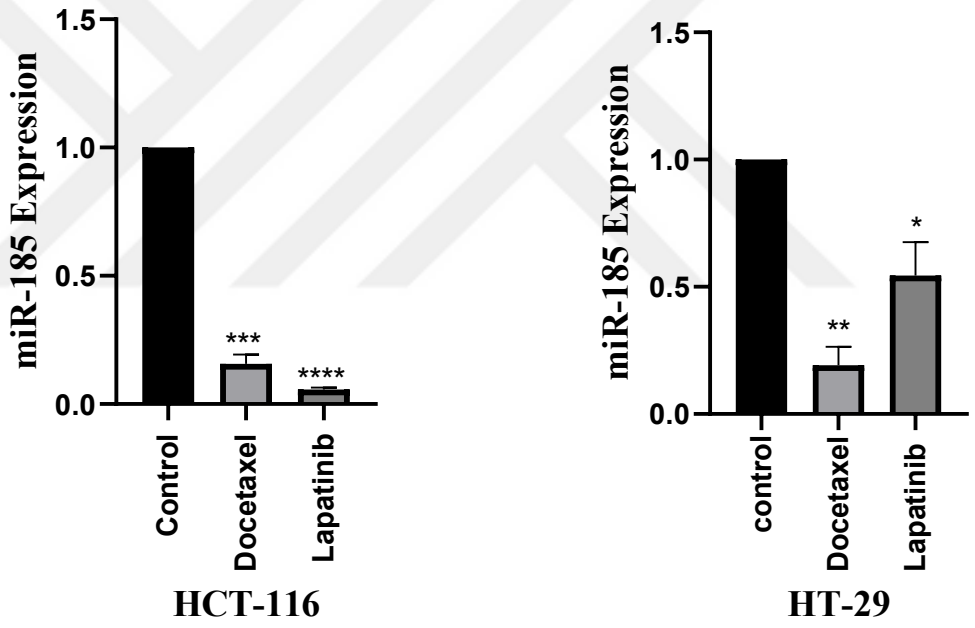

Figure 4.11 levels of miR-185 transcript expression in tumor cells (HT-29 and HCT-116).

After treatment with docetaxel and lapatinib, cells were extracted, RNA was separated, and cDNA was produced. miRNA-185 transcript-specific primers were used in qRT-PCR to generate the data. The acquired data were GAPDH level normalized. The average of three separate experiments is displayed in the graphs' data. For Statistical analysis was performed using a t-test against control groups (****: $P \leq 0.0001$; ***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$).

Effect of docetaxel and lapatinib on miR-1199 Expression in HCT-116 and HT-29 colorectal Cancer Cell Lines.

The expression of miR-1199 was measured after cells were treated with the IC50 values of docetaxel and lapatinib for 24 hours and compared to the control group (no treatment) for both types of cells, HCT-116 and HT-29. Docetaxel increases the expression of miR-1199 transcripts in the colorectal cancer HT-29 cell line by approximately 100% while decreasing it by approximately 80% in the HCT-116 cell line. While using of lapatinib the expression of miR-1199 transcripts in the colorectal cancer HT-29 cell line increased by approximately 40% while decreasing it by approximately 70% in the HCT-116 cell line.

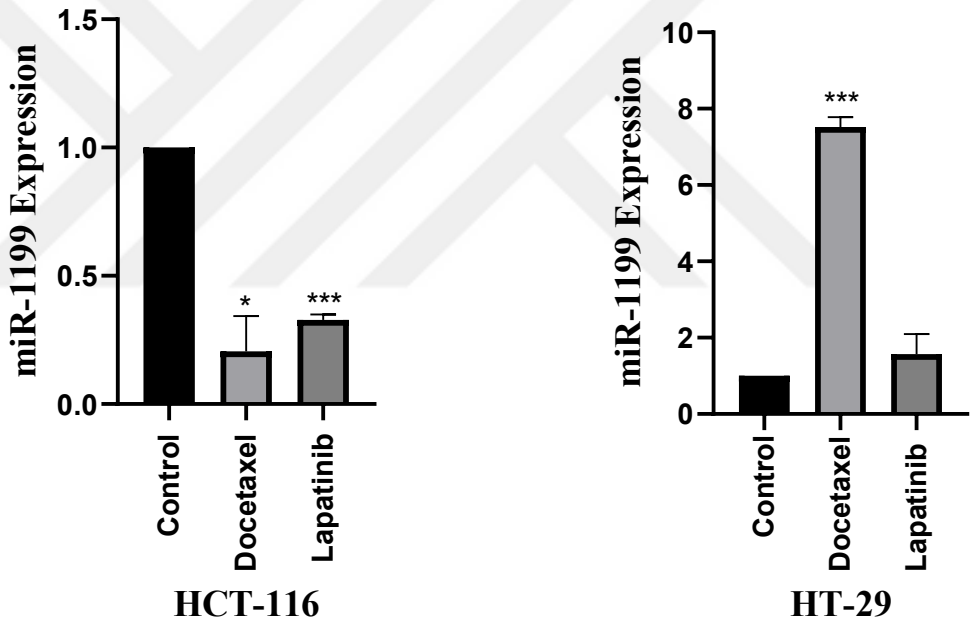

Figure 4.12 levels of miR-1199 transcript expression in tumor cells.

After treatment with docetaxel and lapatinib, cells were extracted, RNA was separated, and cDNA was produced. miRNA-1199 transcript-specific primers were used in qRT-PCR to generate the data. The acquired data were GAPDH level normalized. The average of three separate experiments is displayed in the graphs' data. For Statistical analysis was performed using a t-test against control groups (****: $P \leq 0.0001$; ***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$).

4.3.1. Effect of docetaxel and lapatinib on the Expression of cancer related genes in colorectal cancer cell lines

Effect of docetaxel and lapatinib on FAS Receptors Expression in HCT-116 and HT-29 colorectal Cancer Cell Lines

Here, we investigated the impact of docetaxel and lapatinib on the expression of FAS receptor in colorectal cancer cell lines, specifically HT-29 and HCT-116. Our findings revealed significant alterations in FAS receptor expression compared to the control treatment. In HT-29 cells, treatment with docetaxel and lapatinib at their respective IC50 values led to a substantial increase in Fas receptor expression. Notably, the increase was more pronounced with docetaxel, where the expression compared to the control. Conversely, lapatinib resulted in approximately a 70% increase in Fas receptor expression in HT-29 cells. Contrastingly, in HCT-116 cells, both docetaxel and lapatinib induced a significant decrease in FAS receptor expression. The reduction was approximately 70% when treated with docetaxel and 90% when treated with lapatinib. These findings highlight the differential effects of docetaxel and lapatinib on FAS receptor expression in colorectal cancer cell lines. The distinct responses observed in HT-29 and HCT-116 cells emphasize the importance of considering cell line-sp e cific variations in therapeutic outcomes.

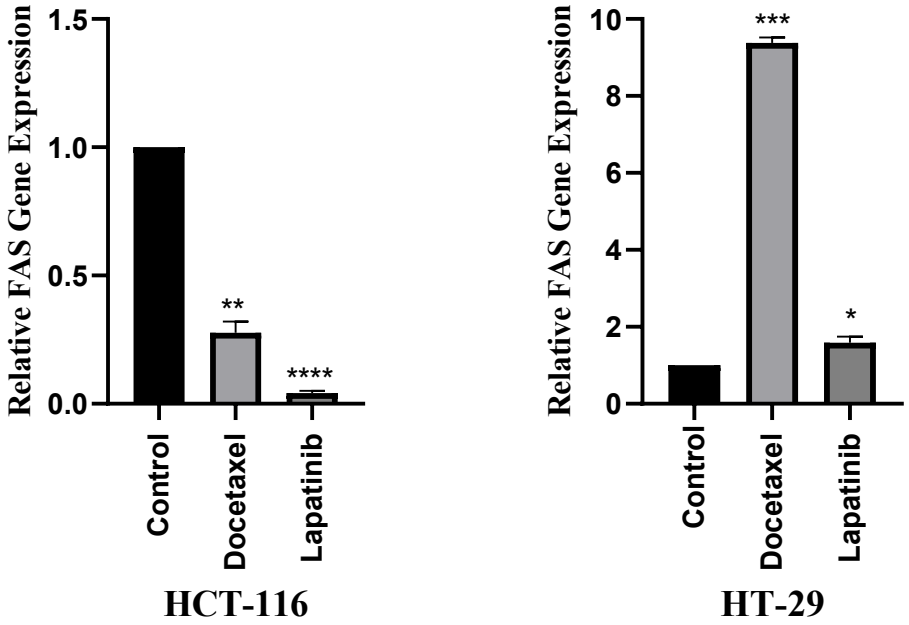

Figure 4.13. Levels of FAS transcript expression in tumor cells.
After treatment with docetaxel and lapatinib, cells were extracted, RNA was separated, and cDNA was produced. qRT-PCR was used to get the data, and particular primers that target the FAS transcript were used. The acquired data were GAPDH level normalized. The average of three separate experiments is displayed in the graphs' data. For Statistical analysis was performed using a t-test against control groups (****: $P \leq 0.0001$; ***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$).

Effect of docetaxel and lapatinib on DR4 Receptors Expression in HCT-116 and HT-29 colorectal Cancer Cell Lines.

we investigated the impact of docetaxel and lapatinib on the expression of the DR4 receptor in colorectal cancer cell lines, specifically HT-29 and HCT-116. The comparison was made against a control group with no treatment. The results demonstrated a significant increase in DR4 receptor expression in HT-29 cells when treated with docetaxel, whereas lapatinib led to a decrease. In contrast, both docetaxel and lapatinib resulted in decreased DR4 receptor expression in HCT-116 cells. Upon treating HT-29 cells with the IC50 values of docetaxel and lapatinib, distinct patterns in DR4 receptor expression emerged. With docetaxel, there was a significant increase compared to the control. However, using lapatinib resulted in a decrease of approximately 20%. In the HCT-116 colorectal cancer cell line, both docetaxel and lapatinib significantly decreased DR4 expression. The reduction was around 90% with docetaxel and 70% with lapatinib.

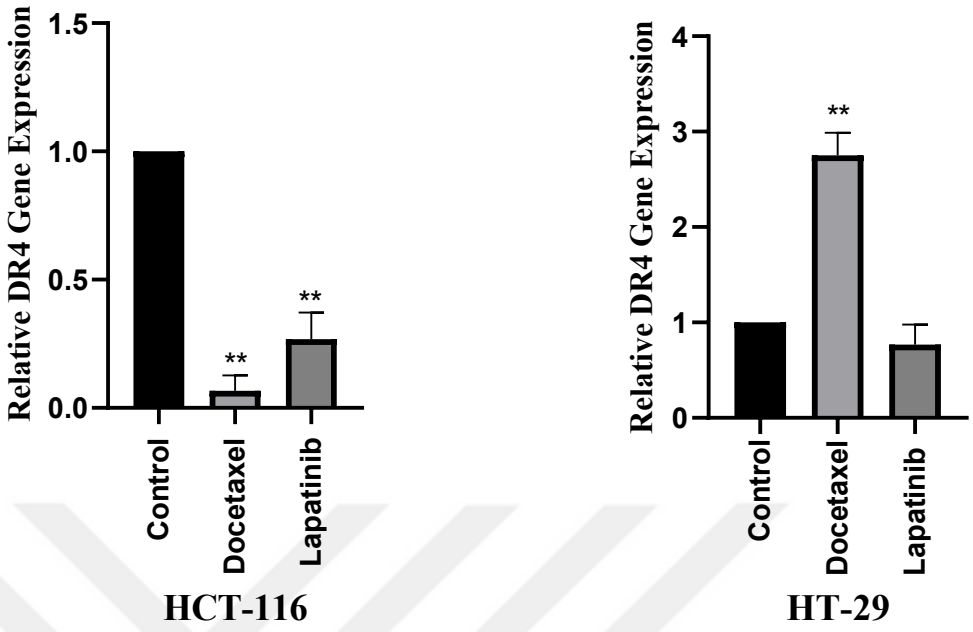

Figure 4.14 levels of DR4 transcript expression in tumor cells.

After treatment with docetaxel and lapatinib, cells were extracted, RNA was separated, and cDNA was produced. DR4 transcript-specific primers were used in qRT-PCR to generate the data. The acquired data were GAPDH level normalized. The average of three separate experiments is displayed in the graphs' data. For Statistical analysis was performed using a t-test against control groups (****: $P \leq 0.0001$; ***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$).

Effect of docetaxel and lapatinib on E2F7 gene Expression in HCT-116 and HT-29 colorectal Cancer Cell Lines

we examined the impact of docetaxel and lapatinib on the expression of the E2F7 gene in colorectal cancer cell lines, specifically HT-29 and HCT-116, in comparison to a control group with no treatment. The results revealed a significant increase in E2F7 gene expression in HT-29 cells when treated with docetaxel, while lapatinib led to a decrease. Conversely, in HCT-116 cells, both docetaxel and lapatinib resulted in decreased E2F7 receptor expression. Upon treating HT-29 cells with the IC50 values of docetaxel and lapatinib, distinct patterns in E2F7 gene expression were observed. Docetaxel induced a significant increase compared to the control, whereas lapatinib resulted in a decrease of approximately 60%. In the HCT-116 colorectal cancer cell

line, both docetaxel and lapatinib significantly decreased E2F7 expression. The reduction was around 10% with docetaxel and 90% with lapatinib.

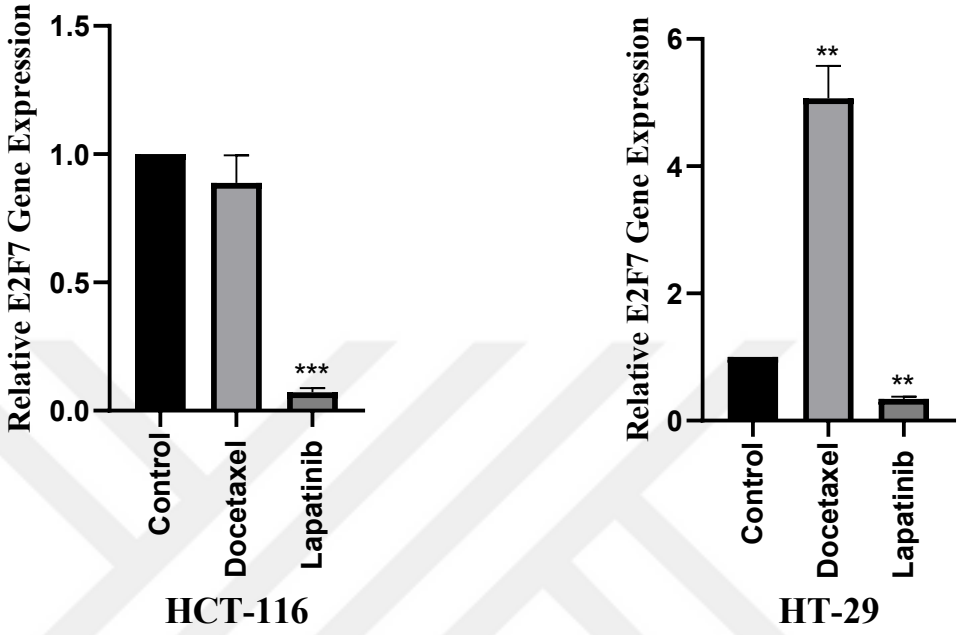

Figure 4.15 Levels of E2F7 transcript expression in tumor cells (HT-29 and HCT-116).

After treatment with docetaxel and lapatinib, cells were extracted, RNA was separated, and cDNA was produced. qRT-PCR was used to get the data, and particular primers that target the E2F7 transcript were used. The acquired data were GAPDH level normalized. The average of three separate experiments is displayed in the graphs' data. For Statistical analysis was performed using a t-test against control groups (****: $P \leq 0.0001$; ***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$).

Effect of docetaxel and lapatinib on EHF Receptors Expression in HCT-116 and HT-29 colorectal Cancer Cell Lines

We examined the impact of docetaxel and lapatinib on the expression of the EHF gene in colorectal cancer cell lines, specifically HT-29 and HCT-116, in comparison to a control group with no treatment. The results revealed a significant increase in EHF gene expression in HT-29 cells when treated with docetaxel, while lapatinib led to a decrease. Conversely, in HCT-116 cells, both docetaxel and lapatinib resulted in decreased EHF

receptor expression. When HT-29 cells were treated with the IC50 values of docetaxel and lapatinib, distinct patterns in EHF gene expression were observed. Docetaxel induced a significant increase compared to the control, whereas lapatinib resulted in a decrease of approximately 60%. In the HCT-116 colorectal cancer cell line, both docetaxel and lapatinib significantly decreased EHF expression, with a reduction of around 90% with docetaxel and 95% with lapatinib.

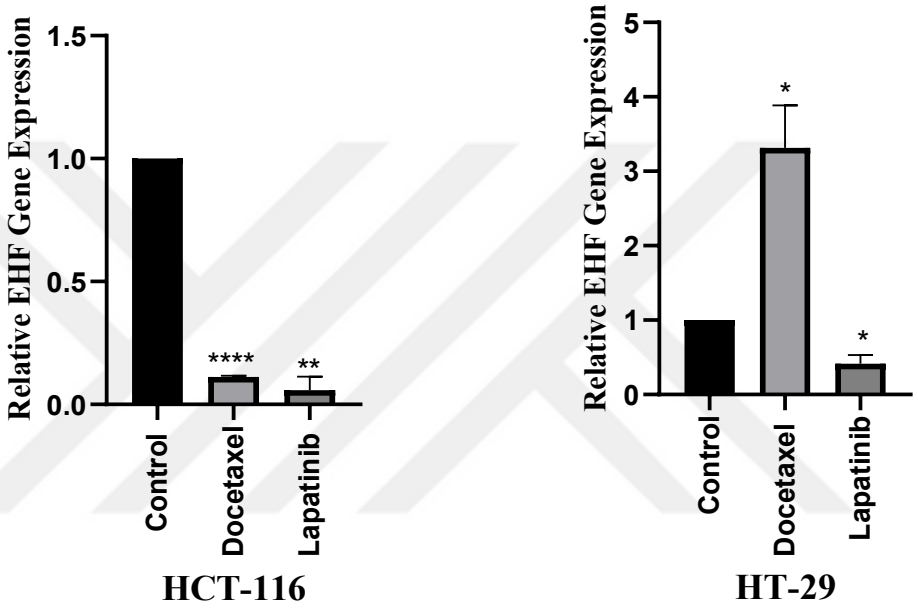

Figure 4.16 EHF transcript expression levels in tumor cells. Following docetaxel and lapatinib treatment, following harvesting the cells, RNA was separated, and cDNA was produced. qRT-PCR was used to obtain the data, and particular primers that target the EHF transcript were used. The acquired data were GAPDH level normalized. The average of three separate experiments is displayed in the graphs' data. For Statistical analysis was performed using a t-test against control groups (****: $P \leq 0.0001$; ***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$).

4.4.miR-595 Overexpression Targeting E2F7 Gene

To explore the regulatory relationship between miR-595 and its putative oncogenic target E2F7, HT-29 cells were transfected with miR-595 mimics. Cells were seeded in 6-well plates and transfected using Lipofectamine RNAiMAX, followed by incubation in serum-containing medium. Total RNA was extracted 24–48 hours post-transfection and subjected to RT-qPCR analysis. Results revealed that miR-595 overexpression significantly reduced E2F7 mRNA levels, confirming its regulatory role. In contrast, treatment with docetaxel alone led to a marked upregulation of E2F7 expression. These results suggest an antagonistic interaction between miR-595 and E2F7, where miR-595 may mitigate docetaxel-induced upregulation of this transcription.

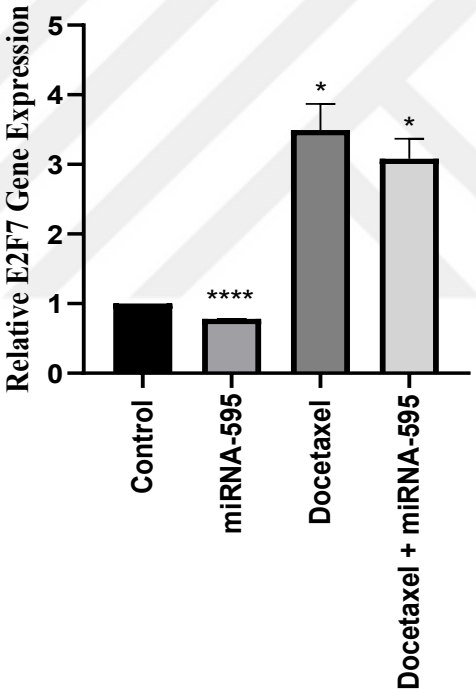

Figure 4.17 Effect of miR-595 Overexpression, Alone or Combined with Docetaxel, on E2F7 Expression. E2F7 expression was quantified by RT-qPCR and normalized to GAPDH. Data represent mean ± SEM of three independent experiments. Data represent the mean ± SEM of three independent experiments. Statistical analysis was performed using Student's t-test. Significance was indicated as: ****$P < 0.0001$, $P < 0.05$.

4. DISCUSSION

In recent years, numerous in vitro methods have been employed to understand and manipulate gene expression in CRC cells, leading to the discovery of novel therapeutic strategies. One of the key challenges in managing CRC is overcoming drug resistance, which strongly affects both disease control and treatment prognosis, in vitro and in vivo. To address this issue, new treatments have been developed using a wide array of strategies.

Docetaxel, a semisynthetic paclitaxel analog, has demonstrated significant efficacy against several human malignancies, including prostate, breast, lung, ovarian and colorectal cancer, owing to its pro-apoptotic and antitumorigenic activities (Fan R et al., 2011). Its mechanism of action involves disrupting microtubules that lead to cell cycle arrest at the G2-M phase and induction of the p53 tumor suppressor and p21 cell cycle inhibitor, ultimately inhibiting growth and inducing apoptosis in cancer cells (Kim IY et al., 2011). Recognizing the potential of docetaxel in colorectal cancer treatment is essential to explore strategies that can enhance its efficacy.

Immunogenic death of a cell causes an immune response after it dies by apoptosis. Death receptors, including FAS (CD95) and DR4 (TRAIL-R1), are particularly involved in this mechanism (Naumann et al., 2011). deregulation FAS and DR4 are common findings in ovarian, CRC cells (Cacan E and Ozmen Z 2020) and this overexpression have been known to decrease chemoresistance in human ovarian cancer (Cacan E, 2017). Profiling of death receptor expression such as FAS may thus aid to promote immunogenicity in tumor cell death.

In this study, our primary objective was to ascertain the specific concentration of Docetaxel affecting colorectal cancer cell viability, namely 0.05 µM, 0.1 µM, 0.5 µM, 1 µM, and 10 µM. Simultaneously, we investigated the impact of lapatinib at concentrations of 0.1 µM, 0.5 µM, 1 µM, 5 µM, and 10 µM. Our findings indicate that varying concentrations of docetaxel induce toxicity in the colorectal cancer cell line. Specifically, higher concentrations of docetaxel significantly decreased cell viability, corroborating previous reports where docetaxel induced apoptosis and growth inhibition in other cancer

models (Yousef, et al., 2020). In contrast, in the case of lapatinib, concentrations of 0.1 µM, 0.5 µM, and 1 µM did not elicit any discernible toxicity in the colorectal cancer cell lines HT-29 and SW-620. However, a modest level of toxicity was observed in the HCT116 cell line. These observations correspond to reported evidence of lapatinib being a dual EGFR/HER2 tyrosine kinase inhibitor which activity relies on the expression pattern regarding these proteins in CRC cells (Ciardiello & Tortora, 2008). Resistance in HT-29 and SW-620 at lower doses could represent differences in HER2 and EGFR expression that may affect lapatinib sensitivity. Overall, therefore our data indicate distinct sensitivities of CRC cell lines to docetaxel and lapatinib and underscore the need for individualized treatment using molecular profiling.This is consistent with clinical observations that combination therapies or tailored treatments improve outcomes in heterogeneous CRC populations (Dienstmann et al., 2017).

We also investigated the impact of docetaxel and lapatinib on pro-apoptotic and oncogenic FAS, DR4, E2F7 and EHF. Docetaxel markedly upregulated FAS, DR4, E2F7 and EHF transcripts in HT-29 cells but decreased their expression in HCT-116 cells which may be due to a genetic background and p53 and KRAS status of the cell lines being used (Gan et al., 2011; Gao et al., 2016). In contrast, lapatinib decreased expression of DR4, E2F7 and EHF transcripts in both cell lines but led to increasing of FAS only in HT-29 cells, consistent with EGFR/HER2 pathway inhibition and crosstalk with apoptotic regulators (Voigtlaender et al., 2018).

Overall, we perceived that docetaxel and lapatinib can influence the expression of death receptors (FAS and DR4) and transcription factors (E2F7 and EHF) in CRC. Docetaxel, in particular, appears to enhance tumor cell sensitivity through the upregulation of death receptor pathways, supporting its continued development alone or in combination with CRC treatment.

After confirming the cytotoxic effects, we examined the changes in expression of several significant microRNAs (miR-1199, miR-595, miR-206, miR-185 and miR-718) involved in CRC tumorigenesis and chemoresistance. miR-1199 was one of them that were upregulated in HT-29 cells upon exposure to docetaxel and also has been reported to act as a tumor suppressor miRNA which inhibits EMT by targeting ZEB1 and stabilizing the

epithelial phenotype in HCC cells (Meyer-Schaller & Christofori, 2017). This upregulation might promote increased chemosensitivity via a suppression of EMT-mediated metastasis and resistance mechanisms, suggesting miR-1199 as a potential biomarker and therapeutic target for CRC.

miR-595 exhibited differential regulation depending on cell type and treatment: it was significantly downregulated in HCT-116 cells exposed to combined docetaxel and lapatinib, whereas it was upregulated in HT-29 cells treated with docetaxel alone. These data suggest a context-dependent role for miR-595, acting as a tumor suppressor in some contexts, consistent with reports in ovarian cancer, and as an oncogene in others, such as glioblastoma (Tian et al., 2015; Hao et al., 2016) The dual role of miR-595 warrants further exploration to clarify its downstream targets and therapeutic potential in CRC.

Similarly, miR-206 increased in response to docetaxel, consistent with its known tumor suppressive functions in CRC by repressing proliferation, migration, and metabolism-related pathways (e.g., hnRNPA1/PKM2) (Zhao et al., 2021; Sun et al., 2020). However, its downregulation by lapatinib suggests a complex, potentially drug-specific regulatory network involved in chemoresistance, highlighting miR-206's potential as a predictive biomarker for treatment response. For miR-718 exhibited opposing regulation between the two cell lines (downregulated in HCT-116 and upregulated in HT-29) suggesting a dual role that may depend on the genetic background and molecular context (Leng, R et al.,2014; Tian L et al.,2018) Its modulation in response to chemotherapy implies a role in chemosensitivity and resistance, meriting further functional studies. For the expression of miR-185 in response to chemotherapy agents in two colorectal cancer cell lines. Our results showed a consistent downregulation of miR-185 in both HCT-116 and HT-29 cells following treatment with docetaxel and lapatinib, suggesting that miR-185 may play a role in the cellular response to chemotherapy. Several studies have reported that miRNAs are aberrantly expressed in CRC tissues or cells, acting as tumor suppressors or oncogenes (Stiegelbauer et al., 2014). Specifically, upregulation of miR-185 has been shown to reduce aggressive features of CRC by directly targeting WNT1, as well as downstream oncogenes MYC and CCND1 (Zhang W et al., 2018). In contrast, miR-185 is downregulated in gastric cancer (GC), promoting cell proliferation and invasion while inhibiting apoptosis and necrosis (Bibi, F et al., 2018). Moreover, miR-185 has been

implicated in regulating chemosensitivity in GC: its downregulation in drug-resistant GC cells increases resistance to chemotherapeutic agents by upregulating multidrug resistance (MDR) genes (Li, Y. et al., 2015), whereas enhanced expression improves chemosensitivity by reducing the expression of the apoptosis repressor with caspase recruitment domain (ARC), both in vitro and in vivo (Li, Q. et al., 2015). These differences are likely due to the distinct genetic mutations and molecular pathways active in each colorectal cancer cell line, which shape their responses to drug and radiation treatments (Calin & Croce, 2006; Jin et al., 2019). Our findings further emphasize that chemotherapy-induced changes in miRNA expression are strongly influenced by these intrinsic molecular characteristics. For instance, treatment with docetaxel and lapatinib led to the upregulation of certain miRNAs in HT-29 cells but downregulation of the same miRNAs in HCT-116 cells. Such variability highlights the complexity of gene and miRNA regulation in cancer and underscores the need for personalized therapeutic strategies tailored to the molecular profile of individual tumors.

We further demonstrated that miR-595 overexpression significantly downregulates the oncogenic transcription factor E2F7 in HT-29 cells. This finding builds upon previous studies in glioblastoma where miR-595 promoted tumor proliferation via suppression of the tumor suppressor SOX7 (Tian et al., 2015). Although SOX7 and E2F7 regulate distinct pathways, both are key regulators of cell cycle progression and tumor growth, indicating that miR-595 may act as an oncogene or tumor suppressor depending on the target and cancer type. E2F7, a transcriptional repressor involved in cell cycle control, DNA replication, and apoptosis (Hao et al., 2016). has been linked to tumor progression and chemoresistance in several cancers including CRC (Chen,K et., al 2024). Our results indicated that miR-595 increases the chemosensitivity of CRC cells through targeting E2F7, thus inhibiting proliferation pathways in CRC cells.

Taken together, these results suggest that miR-595 may serve as a predictive and therapeutic biomarker for chemoresistant patients. Modulating miRNA expression or targeting their downstream effectors could offer novel strategies to improve CRC treatment outcomes.

5. CONCLUSION

Docetaxel and lapatinib induced both direct and indirect changes in the expression of multiple genes involved in critical cellular processes, including proliferation, apoptosis, transcription, translation, oncogenesis, angiogenesis, metastasis, and drug resistance in colorectal cancer (CRC). These findings provide important molecular insights into the pleiotropic effects of these drugs and support the development of mechanism-based targeted therapies, particularly for drug-resistant CRC.

miRNA profiling in CRC cell lines (HT-29 and HCT-116) treated with docetaxel and lapatinib revealed deregulation of several miRNAs compared to untreated controls. miR-595 emerged as a critical regulator, influencing gene expression and chemosensitivity depending on the cellular context. While overexpression of miR-595 showed potential as a modulator of the E2F7 gene, its effects were limited when used alone or in combination with docetaxel, highlighting the need for further investigation.

Overall, these findings emphasize the potential of targeting specific miRNAs and gene pathways as predictive biomarkers and therapeutic strategies in CRC. However, this study is limited to in vitro analyses, and further in-depth investigations are required to establish definitive causal links and validate these results in vivo.

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