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Expression Levels in Various Cell Lines of Natural Antisense Transcripts

DOCTORAL THESIS

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DEPARTMENT OF MEDICAL BIOLOGY AND GENETICS

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Thesis Defence Exam Date: 08.10.2019

Approval of the Health Sciences Institute

I confirm that this thesis satisfies all the requirements as a thesis for the degree of Doctor of Philosophy of (Ph.D.).

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I confirm that this study is a suitable and sufficient study for a "Doctorate" degree. This thesis has been supervised and read by me and has been accepted as a "Doctorate" thesis in terms of scope and quality.

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This thesis has been read and found in the scope and quality andaccepted as a thesis for the degree of Doctor of Philosophy of (Ph.D.).

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DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

AYSHAN RAFAT YASSIN

08.10.2019

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CONTENTS

SYMBOL and ABBREVIATIONS

ADT	Androgen Deprivation Therapy
BEAS-2B	Bronchial Epithelial Cell line
BFGF	Basic Fibroblast Growth Factor
CRC	Colorectal Cancer
CRPC	Castration-Resistant Prostate Cancer
DNMT1	DNA Methyl Transferase1
EGF	Epidermal Growth Factor
H19	Imprinted Maternally Expressed Transcript
HAGLR	HOXD Antisense Growth-Associated Long Non-Coding RNA
HCC	Hepatocellular Carcinoma.
HIF	Hypoxia-Inducible Factors
HOTAIR	Hox Transcript Antisense RNA
LCMT1 AS	Leucine Carboxyl Methyl Transferase 1Antisense
LCK	Leukocyte-Specific Protein Kinase.
LncRNAs	Long Non-Coding RNAs
MALAT1	Metastasis Associated Lung Adenocarcinoma Transcript
MMP	Mixed Member Proportional
mRNAs	Messenger RNAs
ncRNAs	Noncoding RNAs
NAV2AS5	NAV2 AntisenseSystemic Sclerosis (SSc)
NM23	Nucleoside Diphosphate Kinase 23
NPPA	Natriuretic Peptide Precursor A
NSCLC	non-small cell lung cancer
ORF	Open Reading Fragment

PI3K/Akt	Phosphoinositide 3-Kinase
PC	Prostate Cancer
piRNA	Piwi-RNA
PP2A	Protein Phosphatase 2A
PRC2	Polycomb Repressive Complex 2
RIP	RNA Immuno Precipitation
SCLC	Small Cell Lung Cancer
SiRNA	Small Interfering RNA miRNA microRNA
snoRNAs	Small nucleolar RNAs and small nuclear RNAs
TSIX	TranscriptionSpecific Inactive X Chromosome
TUG1	Taurine Up-Regulated 1
VEGF	Vascular Endothelial Growth Factor
WDR5	WD RepeatContainingProtein 5
XIC	X Inactivation Center

LIST of FIGURES

LIST of TABLE

ÖZET

Farklı Hücre Hatlarında Doğal Antisens Transkriptlerin İfade Seviyelerinin Belirlenmesi

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(lncRNA) Uzun kodlanmayan RNA'ların en önemli fonksiyonel özelliği RNA susturulması ve mRNA'nın kontrol ve düzenlenmesidir. LncRNA'lar kanserin bir çok safhasında düzenleyici rol oynar.. Antisens lncRNA'lar bir gen boyunca veya genin bir kısmını içerecek şekilde genin tersi yönünde aktivite göstererek o geni düzenler. Çalışmamızda kullanacağımız TSIX antisens lncRNA'sı XIST genine bağlanarak ifadesini düzenlenmektedir bunada lncNAT denilmekte . TSIX yeni bir regülatör molekuludur özelikle kolejen ekspresyon seviyesinin düzenlenmektedir . HAGLR, farklı kanser türlerinde kanserojenez ve metastaz için kritik bir düzenleyicidir. LCMT-1, Akt proto-onkogen'in negatif regülatörüdür.

böylelikle sonraki çalışmalarla desteklendiği taktirde kanserdeki rolünü anlamamıza yardımcı olacaktır. Çalışmamızda kullanacağımız antisensler; HAGLR, LMCT1, ve NAV2AS5 için henüz yeterli çalışma yapılmamıştır. Ancak literatürdeki bazı veriler kanser gelişimi ile ilişkili olabileceklerini göstermektedir. Bu nedenle çalışmamız sonucunda bu dört antisensin çeşitli kanser ve normal hücre hatında (Beas2B, CRL4010, CRL8798) and kanser (A549, MCF7, MDA-MB-231, CRL2329) hücre hatında ifade seviyelerinin belirlenmesi. İlk aşamada TSIX, HAGLR, LMCT1AS, ve NAV2AS antisens transkriptleri için primer dizaynı yapıldı. Hücre kültürü yöntemi kullanılarak çeşitli kanser ve normal hücre hatları kültüre edildikten sonra RNA izolasyonu yapıldı . İzolasyon sonrası elde edilen RNA'lar normal -PCR metodu ile cDNA'ya çevirilecektir. cDNA'lar kullanılarak tasarlanan primerlerle RT-PCR yapılarak, jel elektroforezinde yürütülerek antisens transkriptlerin çeşitli kanserli ve normal hücrelerdeki ifade seviyeleri nitel olarak belirlendi.

Anahtar kelime: Kanser, HAGLR, LCMT1AS, NAV2AS5, Doğal Antisense RNA'ları.

ABSTRACT

Expression Levels in Various Cell Lines of Natural Antisense Transcripts

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lncRNAs (long non-coding RNAs), with various important molecular and cellular functions and natural antisense transcripts (NATs), complementary to protein-coding or non-coding RNA sequences are important regulators of eukaryotic gene expression drew a great attention recent years to uncover their importance for diagnostic, prognostic and therapeutic purposes because of their dysfunctions that leads to cancer.Earlier NATs were described as lncRNAs. The convergence between NAT and lncRNA determination raised confusion and gradually started to be disappeared with the increasing knowledge. Specific *pcGen (protein-coding Gene)* regulation by their corresponding ncNATs (non-coding NATs) has been reported. TSIX is a new regulator of collagen expression which stabilizes the collagen of mRNA.HOXD-AS1/ HAGLR is a critical regulator for carcinogenesis and metastasis in different types of cancers. LCMT-1 is a negative regulator of Aktproto-oncogene. Different tissue-specificity of NAV2AS5 wasobserved from human tissue samples. Antisense transcripts that bind to long noncoding RNAs are called lncNATs.Among the potential clinical utilities of lncNATs, are of high interest for cancer treatment, even though the biological significance remains still under scientific investigation with major key questions yet to be elucidated.Wehypothesized that lncNATs, TSIX, HAGLR, LMCT1AS, and NAV2AS5 might have important roles for several tumorigenic processes. To explore their roles, ATCC normal (Beas2B, CRL4010, CRL8798) and cancer (A549, MCF7, MDA-MB-231, CRL2329) cell lines were subjected to examine through RNA isolation, cDNA conversion,andSemi-quantitative by using of agarose gel and ImageJ program, alsousing of quantitative RT-PCRfor expression analyses. TSIX, HAGLR, LMCT1AS, and NAV2AS5 genes have differential expression patterns in both normal and cancer cell lines including lung and breast. The obtained results indicate their importance for biological processes in cancer.

Keywords: Cancer, HAGLR, LCMT1AS, NAV2AS5, Natural Antisense RNAs.

1. INTRODUCTION

Long noncoding RNAs (lncRNAs) are a novel class of RNA molecules defined as transcripts which are longer than 200 nucleotides that lack protein-coding potential. They constitute a major, but still poorly characterised part of the human transcriptome. They are important regulatory molecules involved in various cellular processes (1).

Some lncRNAs can have a highly specific expression in particular types of cancer making them a promising tool for diagnosis. It is becoming increasingly clear that many lncRNAs are deregulated in cancer and some of them can be important drivers of malignant transformation(2).

lncRNAs are thought to have various functions other than RNA silencing. Researchers tried to evaluate the expression of lncRNAs in patients with systemic sclerosis (SSc) and determined whether lncRNAs control collagen expression in dermal fibroblasts (3).

Non-coding RNAs have many different classes of small or long noncoding RNA with their properties and functions. Small non-coding RNA includes microRNAs, small nucleolar RNAs (snoRNAs) and small nuclear RNAs snRNAs.lncRNAs can be located in both nuclear and cytosolic fractions(3).

Non-coding RNAs (ncRNAs) have gained another dimension with new findings. Long-coding RNAs are a broad and diverse class of transcriptional RNA molecules that do not encode proteins with more than 200 nucleotides in length. Many lncRNAs, including transposons, pseudogenes, and simple repetitions that are biologically important functional regulators, which they are transcribed from genomic regions called 'trash' (2, 3). LncRNAs are becoming more and more important as a research topic related to cancer. So far, many lncRNA molecules have been identified that have roles in cancers. Some lncRNAs act as tumor suppressors, while others behave like oncogenes. It is reported that many lncRNAs such as MALAT1, H19, and HOTAIR which act as oncogenes in many cancers such as lung cancer, colon cancer, liver cancer, breast cancer,and another type of cancers (4). There are many lncRNAsthat act as tumor suppressors genes. MEG3 gene acts as a tumor suppressor in many different types of cancer (5). lncRNAs act by enhancing or decreasing expression levels by binding to noncoding or protein-encoding RNAs. Natural antisense transcripts

(NATs), complementary to protein-coding or non-coding RNA sequences are important regulators of eukaryotic gene expression. Natural antisense transcripts that bind to lncRNAs are called long noncoding lncNATs. There are not enough studies yet for a new class of lncNATs. TSIX is a new regulator gene of collagen expression which stabilizes the collagen mRNA, XIST and TSIX transcription dynamics is regulated by the X-to-Autosome Ratio and stabilizes the transcriptional factor (5). Dysregulation of lncRNAs is discovered in various tumor tissues and cancer cells where they can serve as oncogenes or tumor suppressors. Long non-coding RNA HOXD-AS1 is involved in the development of different cancers including neuroblastoma and breast cancer. However, the role of HOXD-AS1 in lung cancer yet to be elucidated. HOXD-AS1 regulates the proliferation ofprostatecancer (6). We hypothesized that TSIX, HOXD-AS1/HAGLR, LCMT1AS, and NAV2AS5 antisense might be regulated by each other for several biological processes in cancer. The other hypothesis for selecting these four types of genes in our study is that all of them may have roles as tumor suppressors. We checked TSIX, HOXD-AS1/HAGLR, LMCT1AS, and NAV2AS5 antisense transcripts in normal cells (Beas2B, CRL4010, CRL8798) and cancer cells (A549, MCF7, MDA-MB-231, CRL2329). These are commercially available cell lines from the ATCC. Until now, it is still a matter of debate whether TSIX is acting as an oncogene or as a tumor suppressor. In many biological processes, there are many lncRNAs targeting proteins that bind to DNA (7). LncRNAs act with proteins that bind to DNA to regulate the transcription of DNA epigenetically. The interaction of TSIX with long coding RNAs is still open to research. Therefore, this study aimed to find the interactive role between lncRNA TSIX, HOXD-AS1/HAGLR, NOV2AS5, and LCM1AS in breast and lung cancers. Also, we would like to address the respective role of TSIX, HOXD-AS1/HAGLR, NOV2AS5, and LCM1AS in both breast and lung cancer.This study will open a new avenue in lung & breast cancer therapy by understanding their oncogenic or tumor-suppressive role and also it will be helpful to find new therapeutic targets (8).

2. GENERAL INFORMATION

2.1.1. Long non-coding RNAs are a major sequence effect on the health problem in all countries and predominantly affects are different. It is the most common type of gene regulation in women and manresponsible from most of the cancer-related to gene expression and regulate sequence of non coding RNA from different population a large and diverse class of transcribed RNA molecules with a length of more than 200 nucleotides that do not encode proteins (or lack > 100 amino acid open reading frame).Many studies have been done about the types and biology of cancers for many years. Studies have been showed that the cancer cell loses their death mechanisms and behaves like pseudo embryonic cells.LncRNAs are thought to nearly 30,000 cause totranscripts in humans. Hence lncRNA transcripts account for the major part of the non-coding transcriptome. lncRNA discovery is still at a preliminary stage. There are many different types oflncRNA databases, which are organized and centralized through RNAcentral (9).

The total human genomes consist of more than 2milion of linear DNA that is inserted into a three-dimensional structure in the nucleus of each cell (9). The central doctrine of molecular biology suggests that the flow of genetic information is from DNA to RNA and from RNA to protein. However, in the last decade, this dogma has gained new dimensions with the discovery of noncoding RNAs (ncRNAs). The Encyclopedia of DNA Elements (ENCODE) project has demonstrated that at least 75% of the human genome encodes RNAs and only 3% of them constitute protein-encoding genes. One of the greatest biological discoveries in the last decade has been the discovery of non-coding RNAs that play a key role in many biochemical, cellular processes (e.g., RNA repair), and have great prominence in human health and disease(10).

2.1.2.Short coding RNAs

With the discovery of a few dozen short ncRNAs, the shortest ncRNAs were the most abundant RNA class. These include well-defined hamart ncRNAs carrier RNA (tRNA) and some ribosomal RNA (rRNA), splicing RNAs and small nuclear RNAs (snRNAs) required for basic aspects of cell biology, transcription initiation RNAs, short RNAs associated with

promoters, RNAs derived from 3'UTR, and various RNAs that have recently been observed associated with protein-encoding gene transcription (11).

2.1.3. Small interfering RNAs (siRNAs)

Small interfering RNAs are mediators of mRNA degradation in the RNA interference (RNAi) process (11). They are produced from long dsRNAs of exogenous or endogenous origin. RNAi is a post-software gene silencing mechanism that is triggered by the appearance of double-stranded RNA (dsRNA) in the most eukaryotic cells (11, 12). The dsRNA is first processed by Dicer into small RNA pairs of length 21-23nt, which is characterized by 2-nt 3'-hydroxyl protruding ends called siRNA pairs and 5 'phosphatidylchain. Subsequently, a strand of the siRNA double strand is incorporated into the RNA-induced silencing complex (RISC) to direct sequence-specific cleavage of the substrate mRNA or complementary target (12).

Synthetic siRNAs are usually produced by solid-phase chemical synthesis methods that allow for the production of pure, stable and easily modified siRNAs. Small double-stranded siRNAs are transferred to the cells into which the guide strand is loaded into the RISC. This activated protein-nucleic acid complex can silence the gene by providing excellent complementarity, dividing, and breaking down the mRNA by binding to a single target mRNA sequence (Figure 2.1) (12).

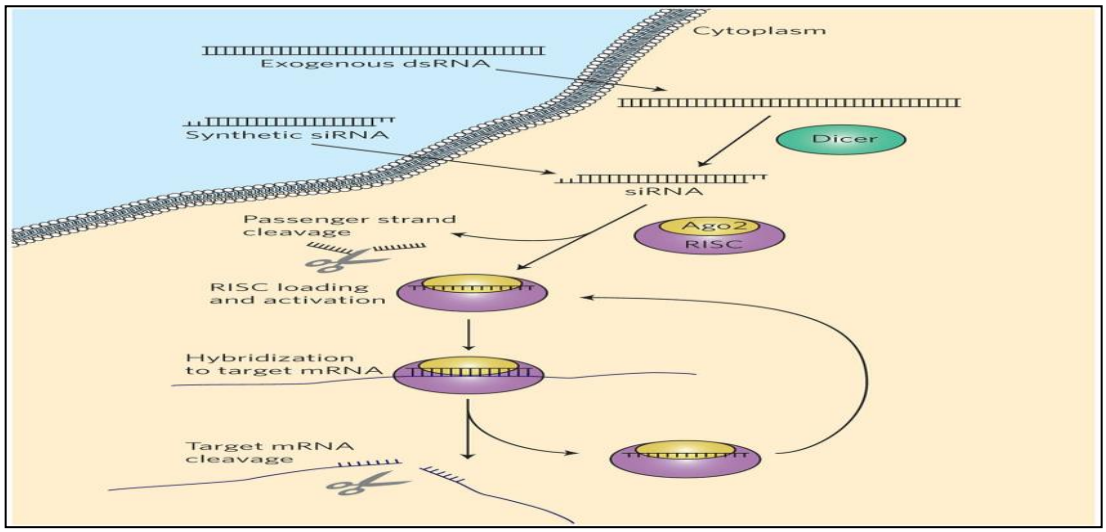


Figure 2.1. RNA intervention. The long dsRNA is entering the cytoplasm(12).

2.1.4.LncRNAs and Epigenetic:

LncRNAs have been implicated in the control of gene expression through the recruitment of epigenetic modifiers at specific genomic loci. In eukaryotic chromatin, epigeneticregulation is conveyed by covalent modifications of DNA (methylation,hydroxymethylation),modifications of histone tails (acetylation,methylation, phosphorylation, and ubiquitinylation), beside the incorporation of various histone variants. These modifications locally change chromatin organization and regulate gene expression without any changes in the DNA sequence. Severalevidencesindicate that lncRNAs, acting as guides targeting enzymes involved in chromatin modifications(13).

2.1.5.Functions of LncRNAs

Recent studies mentioned several regulatory paradigms and they have been defined for how long ncRNAs work. The promoter region of the RNA that is not encoded upstream, the software affects the expression of the negative or positive downward gene expression respectivelyby suppressing chromosomal reshaping and the coming of the RNA Pol II (14). An antisense transcript has the ability to hybridize with overlapping sense transcripts and prevents recognition of fusion sites by the spliceosome. Hence, it causes alternative splicing (14). Alternatively, hybridization of sense and antisense transcripts allows Dicer to produce endogenous siRNA. It can serve as a functional structural component that permits the formation of a larger RNA-protein complex, or it can alter the location of the protein in the cell, which can alter the activity of an unencoded transcript protein by binding specific protein mates. They can be processed to produce long ncRNAs, miRNAs, piRNAs, and other poorly characterized small RNAs(Figure 2.2) (15).

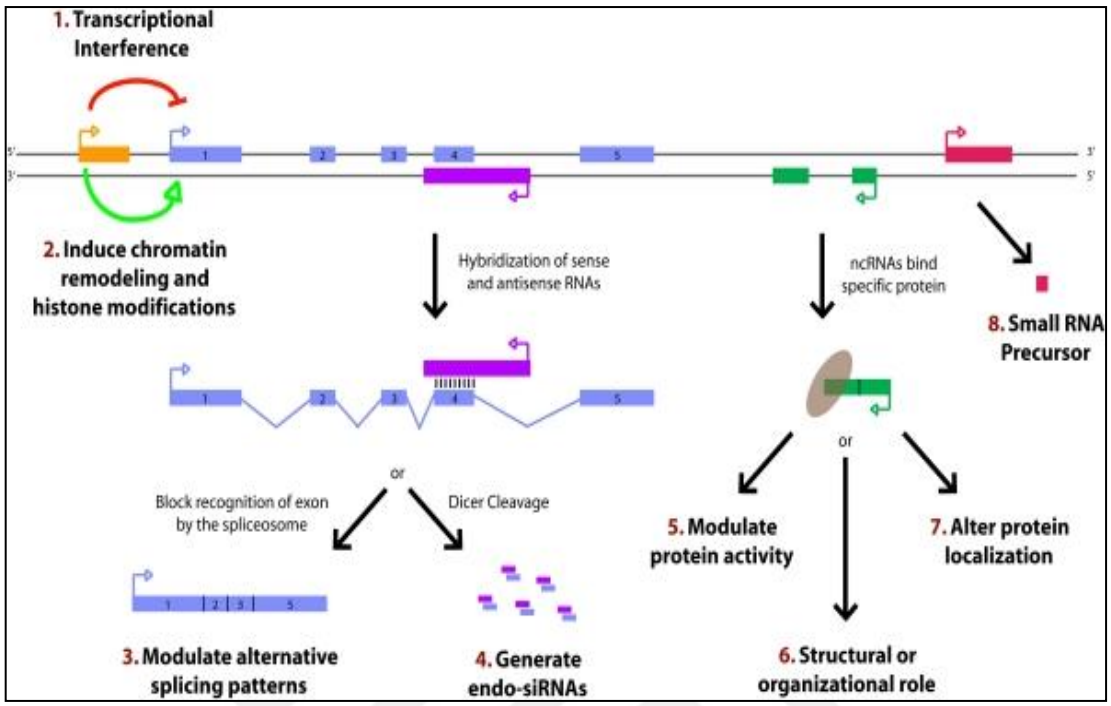


Figure 2.2The paradigm that shows how long ncRNAs work (15).

2.1.6.LncRNA mechanisms

Recently, many attempts have been made to categorize various types of molecular mechanisms that may be involved in lncRNA function. LncRNAs can be identified as one or more of the following four modes (Figure 2.3) (15).

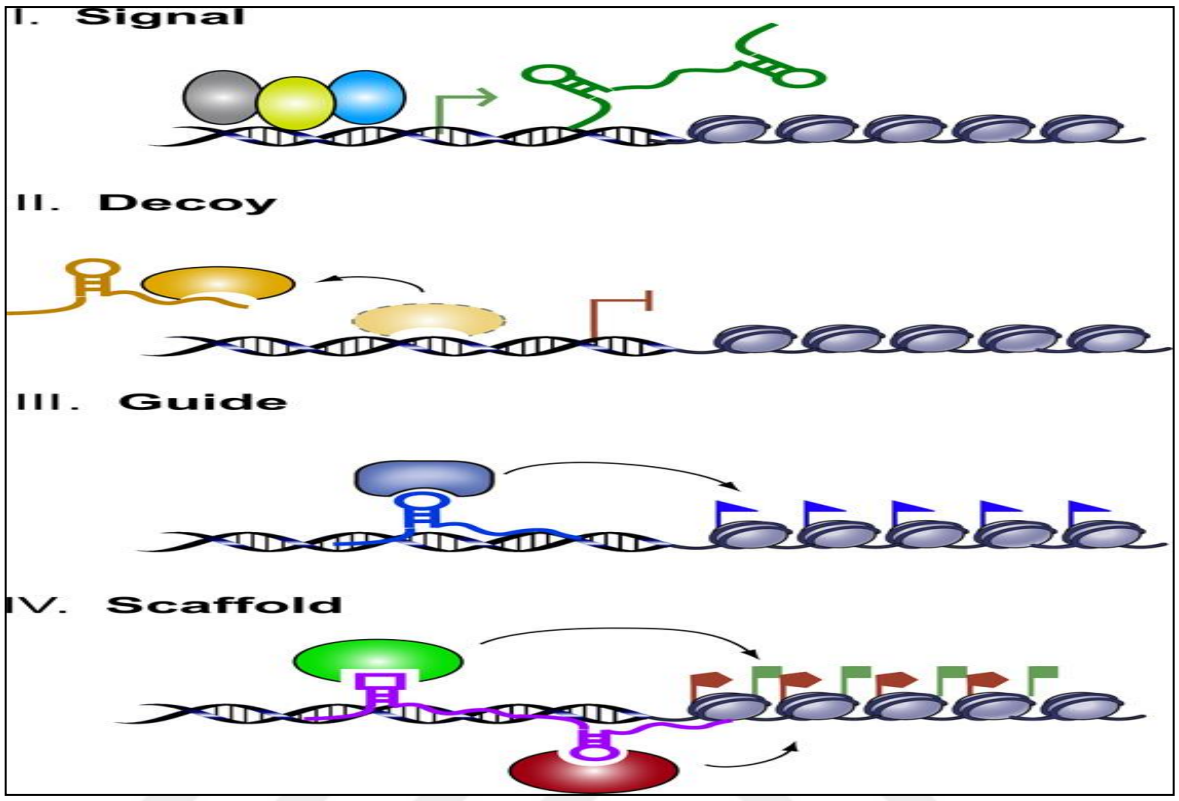


Figure 2.3. Schematic representation of four models of the LncRNA mechanism.The model I: As markers, lncRNA expression may show spatial and temporal gene regulation of the combinatorial effects of transcription factors (color ovaries) or signaling pathways. Model II: As a breakdown, LncRNAs, transcription factors and other proteins can titrate out of chromatin. Model III: As guidebooks, LncRNAs can collect chromatin-converting enzymes into distant target genes in cis or transposition. Model IV: as a scaffold, LncRNAs can combine many proteins to form ribonucleoprotein complexes (15).

2.1.7. Regulation of gene expression by lncRNAs

lncRNAs play an importantrole in modulating genetic networks and signal transduction pathways during development and deregulation of its effect leads totheformation of different cancers.lncRNAs modulate gene expression by specifically associating on the promoters for enhancers of the target gene (Table 2.1 & 2.2) (16).

Table 2.1 Type of long noncoding RNAs

Long noncoding RNA	**Symbol**
Long intergenic noncoding RNA	Linc RNA
Long intronic non –coding RNA	Linc RNA
Natural antisense transcript	NAT
Promoter-associated long RNA	PAIR
Promoter-upstream transcript Repetitive element	PROMPT
Transcript ultraconserved region.	T-UCR
Enhancer-like non –coding RNA	eRNA

Table 2.2 List of lncRNAs dysregulated in cancers

No.	Name	Cancer type	Biological function	Molecular function
1	HOTAIR	Breast,hepatocellular, colorectal	Promotes invasion and metastasis	Scaffold (PRC2, LSD1)
2	ANRIL	Prostate,leukemia, melanoma	Suppresses senescence via INK4A	Scaffold (PRC1, PRC2)
3	MALAT1	Lung, prostate,breast	Regulates alternative splicing of pre-mRNA	Splicing nuclear paraspeckle
4	PCAT-1	Prostate	Promotes ,proliferation, inhibits BRCA2	Scaffold (PRC2)
5	TUC338	Hepatocellular	Promotes,proliferation	Unknown
6	UC. 73a	Leukemia, colorectal	Promotes,proliferation	Unknown
7	SPRY4-IT1	Melanoma	Promotes proliferation and invasion	Unknown
8	ncRNA	Neuroblastoma	Promotes,proliferation	Unknown
9	PRNCR1	Prostate	Promotes ,proliferation	Unknown
10	H19	Breast, hepatocellular	Promotes,proliferation	Unknown

2.2.1.Cancer

Cancer is one of the leading health problems,lung cancer among male and breast cancer among females is one of the common cancers in humans. In spite of the developing treatment methods, lung and breast cancer cannot be treated effectively. Therefore, the identification of novel biomarkers that will be important in the treatment and diagnosis progress of breast cancer is becoming a great interest.Cancer is a leading cause of death throughout the world,from a total of 58 million death worldwide in 2005,cancer accounts for 7.6 million or 13%of all death (16).The main type of cancer leading to overall mortality is lung(1.3million deaths/year) stomach (almost 1 million deaths/year),liver(662.000 death/year),colon (655,000death/year)and breast cancers (502,000 death/year). Death from cancer in Australia is increasing, with the total mortality rising from 34,857 in 1996 to 37,214in 2001 and to 39,200 in 2006 (16).

Cancer is a genetic term that encompasses more than 100 diseases that can affect any part of the body.Theterm cancer only applied to malignant tumors, therefore by definition,all cancer is malignant tumors (17).Tumours may be either benign or malignant and are believed to emerge only when immune surveillance fails the two important differences between benign and malignant tumors invasion and spread (18).

Breast cancer is a major health problem in all countries and predominantly affects the women population. It is the most common type of cancer in women and responses from most of the cancer-related death among women population(18). In the United States, it is estimated that 255,180 new cases of breast cancer and 41,070 breast cancer-related deaths will occur. Also, according to 2014 cancer statistics in Turkey, breast cancer (24.9%) is the most common cancer of women in all age groups (19).

2.2.2. Metastasis

Metastasis is responsible for more than 90 % of patients' deathis composed of more than the third most common malignancies worldwide. Long noncoding RNAs are an important role in transcribed RNA molecules morethan 200 nucleotides in the sequence. Cause development of the genome sequencing technologies, they have been gained more attention. Suggested that abnormal expression of lncRNAs cause to diseases are involved in various biological

functions such asmetastasis, apoptosis,proliferation, and differentiation by effectiveof epigenetic, transcriptional or post-transcriptional,splicing,and regulators(20).

The amount of RNA or long RNA without coding protein ability was revealedit is advancedwith genome sequencing technique. These ncRNAsconsist of small nucleolar RNAs (snoRNAs),microRNAs (miRNAs), piwi-interacting RNAs (piRNAs),and small interfering RNAs (siRNAs).Long noncoding RNAs are non-coding transcripts of more than 200 nt in length, and the majority of them are located in nuclear(20).

Tumourigenesis and cancer progression have critical roles in lncRNAs and play an important rolein it. Their function relatedto tumor suppressors and oncogenes in diversebiological processes, such as imprinting, apoptosis,epigenetic regulation, transcriptional, translational regulation, cell cycle, splicing, cell development, and differentiation (20, 21).

Cancer metastasis is a pathological process that affects the rate of morbidity and mortality among cancer patients. In a patient, one may feel that cancer has suddenly fallen and that their lives have changed immediately.However, diagnosis follows a culmination of yearspossibly decades of alterations occurring at the genetic, molecular, cellular, tissue, and organismal levels. Fortunately, tumor formation and especially metastatic processes are extremely inefficient, and only small parts of the cells from a tumor massactually overcome the many hurdles to grow at a distant site (21).

Metastatic disease is the main cause of death from cancerand limited success in reversing the progression of immunotherapy and chemotherapy. Data from mouse models suggest that the recruitment of immunosuppressive cells to tumors protects metastatic cancer cells from surveillance by killer cells, which nullifies the effects of immunotherapy and thus establishes metastasis(21).

2.2.3.Angiogenesis in cancer

Angiogenesis, the growth of new blood vessels, is an important natural. The procedure is necessary to cure the wounded and correct the flow of blood to the tissues after injury or insult.Angiogenesis therapiesproduced to "turn on" new capillary growth are revolutionizing medicine by providing a unified approach for treating crippling and life-threatening

conditions. Currently, more than 200 biotechnology, genomics, and medical device companies and every major pharmaceutical company are racing to develop new angiogenesis-based medicines.The new growth in the vein web has been important since proliferation, as well as metastatic spread, of cancer cells which depends on an adequate supply of oxygen and nutrients and the removal of waste products. New blood and lymphatic vesselsform through processes called angiogenesis and lymphangiogenesis, respectively. Angiogenesis is regulated by both activator and inhibitor molecules(22).Cancer can spread to adjacent or distant organs, which makes it life-threatening. Tumor cells can penetrate blood or lymphatic vessels then circulate through the intravascular stream which leads to proliferating at another site for the metastatic spread of cancer tissue.Therefore, the growth of the new vascular network is important. The processes whereby new blood and lymphatic vessels form are called angiogenesis and lymphangiogenesis, respectively(22). MALAT1 was also proved to promote angiogenesis in vitro and vivo. MALAT1 wasactivated angiogenesis byaccumulating lncRNAs, including H19, lincRNA-p21, TUG1, and HOTAIR was proved to be involved in angiogenesis in different cancers(23).

Induction of angiogenesis when cancer cells grow and multiply, tumor size and mass increase. If the cancer cells do not gain the ability to stimulate angiogenesis, this growth process will be limited by the natural diffusion limit of oxygen and nutrients. The formation of new blood vessels allows not only nutrients and oxygen uptakebut also the elimination of metabolic waste of tumors and entry into the hematogenous metastatic process. The angiogenic switch "angiogenic switch" is also active in tumor progression. This leads to the continual formation of new blood vessels that help maintain tumor growth. Tumor cells stimulate pro-angiogenic factors to suppress or suppress anti-angiogenic stimuli (23).

2.2.4. Role of LncRNAs in cancer

The identification of novel biomarkers that will be important in the treatment and diagnosis of cancer is of great interest,several Antisense transcription like(TSIX, HAGLR, LCMT1AS1, and NOV2AS5) long non coding RNAs effect directly on the tissue type because deregulation of genes cause to degradation protein and translation factor and defect cell to develop cannot differentiation and proliferation cell its key players in genetics and

pathogenesis of cancer and their dysfunction is closely associated with cancer development, progression, and metastasis(23,24).

In the developed countries breast cancer diseases are the leading causes of death (34%), followed by lung cancer (23%). Cancer is a group of diseases characterized byunregulated cellgrowth and the invasion and spread of cells from the site of origin, or primary site, to other sites in the body. Only 10% of cancer cases are inherited, 70% are sporadic, that is, the mutations are not inherited but occur during the life course of an individual (24). Twenty percent of cancer resultsin pathogenic infections, mainly viruses. The tumor is not synonymous with cancer. A tumor can be benign, pre-malignant, or malignant, whereas cancer is by definition malignant. Earlier many scientists were confident that science found the final answer on cause of tumorigenesis: cancer is the result of cumulative mutations that change proteins encoded by cancer-related genes(24).

Many studies have been done about the types and biology of cancers for many years. Studies have been showed that the cancer cell loses their death mechanisms and behaves like pseudo embryonic cells.This is not actually true. It is also possible to see the died and calcified regions in cancerous cells and tissues. However, tumor mass is formed due to the higher cell division of somatic cells compared to cell death(25).Cancer results from the deregulation of expression and function of proto-oncogenes and tumor suppressor genes. Proto-oncogenes encode for proteins involved in cell proliferation, differentiation, cell cycle, and cell death mechanisms (25).

Mutations and gene expression changes in these genes lead to the dominant functional properties of these genes in somatic cells.In contrast, tumor suppressor gene products inhibit cell proliferation by suppressing the synthesis of proteins necessary for cell cycle and keeps cells in the differentiated phase (26). Mutations in tumor suppressor genes lead to the deregulation of the cell cycle and the possibility of cell proliferation and tumor development increases (26, 27).Another mechanism that drives tumor formation in the presence of genomic instability in clone cells and deregulation of DNA repair mechanism. Mutations that can occur in DNA repair mechanisms also trigger cancer development(26).Cell proliferation and differentiation, and death of quiescent differentiated cells maintain organ and tissue integrity as a result of a series of genetically controlled functions. The balance between cell

proliferation and death changes towards tumor development due to alterations in the expression of proto-oncogenes and tumor suppressor genes. Among these genes, Myconcogene regulates (26).

Most cancers have mutations in proto-oncogenes, the normal genes involved in the regulation of controlled cell growth. These genes encode proteins that function as growth factors, growth factor receptors, signal-relaying molecules, and nuclear transcription factors proteins that bind to genes to start antisense transcription. When the proto-oncogene is mutated or overregulated, it is called an oncogene and results in unregulated cell growth and transformation. At the cellular level, only one mutation in a single allele is enough to trigger an oncogenic role in cancer development. The chance of getting cancer is increasing by the increasing age (27).

Cancer is a disease class that characterizes the growth of uncontrolled cells. It is an important public health problem all over the world. For example, in the US, the likelihood of developing life-long cancer is 44% in males and 38% in females (27, 28). Each year, the American Cancer Society estimates new cancer cases and mortality in the United States and estimates the number of deaths and the latest data on cancer incidence, mortality, and survival. In 2016, 1,685,210 new cancer cases and 595,690 cancer deaths were estimated to occur in the United States (28). According to the World Health Organization (WHO), the number of new cancer cases is expected to increase by about 70% over the next 20 years. The most common areas of cancer in males are lung, prostate, colon, rectum, abdomen, and liver, while females are classified as breast, colon, rectum, lung, cervix and stomach cancers. Although cancer does not include a heterogeneous group of diseases, one of the characteristic and associative traits is the formation of abnormal cells that grow beyond their natural borders. Since tumor formation is a very gradual process, normal cells gradually progress to the neoplastic stage, during which time they acquire special capacities that allow them to be tumorigenic(29). Cancer is a genetic disorder caused by abnormalities in genes that control vital biological processes such as cell growth and division. Genetic changes that contribute to cancer affect three basic types of genes such as proto-oncogenes, tumor suppressor genes, and DNA repair genes(29).

In the developed countries breast diseases are the leading causes of death (34%), followed by cancer (23%). Cancer is a group of diseases characterized by unregulated cell growth and the invasion and spread of cells from the site of origin, or primary site, to other sites in the body. Only 10% of cancer is inherited, 70% is sporadic, that is, the mutations are not inherited but occur during the life course of an individual (30). Earlier many scientists were confident that science found the final answer on cause of tumorigenesis: cancer is the result of cumulative mutations that change proteins encoded by cancer-related genes(31).

Most cancers have mutations in proto-oncogenes, the normal genes involved in the regulation of controlled cell growth(32). These genes encode proteins that function as growth factors, growth factor receptors, signal-relaying molecules, and nuclear transcription factors (proteins that bind to genes to start transcription)(32, 33). When the proto-oncogene is mutated or overregulated, it is called an oncogene and results in unregulated cell growth and transformation(33). At the cellular level, only one mutation in a single allele is enough to trigger an oncogenic role in cancer development(33, 34, 35). The chance of getting cancer is increasing by the increasing age (34, 35).

Most cancer susceptibility genes are tumor suppressor genes. These genes, under normal circumstances, suppress cell growth. Some do so by encoding transcription factors for other genes neededfor slow growth(36). For example, the protein product of the tumor suppressor gene TP53 is called p53. It binds directly to DNA and leads to the expression of genes that inhibit cell growth or trigger cell death(36). Other tumor suppressor genes code for proteins that help control the cell cycle(36, 37). Both copies of a tumor suppressor gene must be lost or mutated for cancer to occur. A person who carries a germline mutation in a tumor suppressor gene has only one functional copy of the gene in all cells (37). For this person, loss or mutation of the second copy of the gene in any of these cells can lead to cancer(37).In 1971, Alfred Knudson (38), proposed the two-hit hypothesis to explain the early onset at multiple sites in the body of an inherited form of cancer called hereditary retinoblastoma(38). Inheriting one germline copy of a damaged gene present in every cell in the body was not sufficient to enable this cancer to develop. A second hit (orloss) to the good copy in the gene pair could occur somatically, though, producing cancer(39). This hypothesis predicted that the chances for a germline mutation carrier to get a second somatic mutation at any of

multiple sites in the body cells were much greater than the chances for a noncarrier to get two hits in the same cell. Therefore, accordant to the two-hit hypothesis, tumor suppressors act recessivelyat the phenotypic level both alleles must be mutated or lost for cancer to develop(40).In hereditary cancer syndromes, individuals are called heterozygous (having one or more dissimilar gene pairs) because they start life with a mutation(inherited from one of the parents) in one of the alleles linked to cancer susceptibility, but it is balanced by a normal counterpart(40). These individuals are predisposed to cancer because all their cells have already sustained the first hit to cancer-linked genes. If the critically needed normal suppressor gene that balances this germline mutation is lost at some time point during an individual's life, a condition called the loss of heterozygosity occurs (40, 41).

Oncogenes (tumor-causing genes) are cellular genes that can trigger uncontrolled cell proliferation if their sequence is altered or their expression is incorrectly regulated(28). Oncogenes were originally identified in RNA tumor viruses (retroviruses) as genes (v-onc) that could transform cells into an altered state of control of cell proliferation, often resulting in a tumor, mainly in chicken, mice, and rats(41). More than 20 different viral oncogenes are known to have a counterpart in normal cells (c-onc), called proto-oncogenes or cellular oncogenes(41, 42). Cellular oncogenes encode proteins that are required at defined sites throughout the cell where they regulate the ordered progression through the cell cycle, cell division, and differentiation(42). In virtually every case where their functions are known, oncogenes lie along the signaling pathways by which cells receive and execute growth instructions. The mutations that activate these genes are either structural mutations that lead to the constitutive activity of a protein without an incoming signal (e.g., the protein kinases or Ras) or regulatory mutations that lead to the expression of the gene at an elevated level or at the wrong place and time (e.g., growth factors or transcription factors) shows at (Figure 2.4)(43).

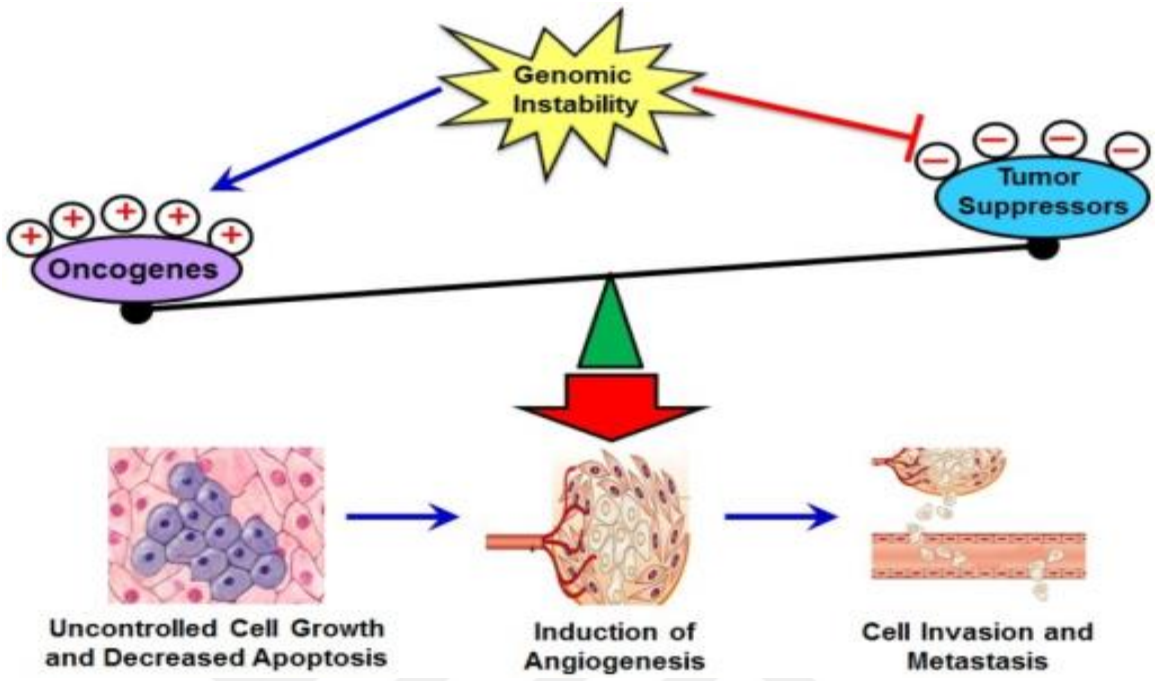


Figure 2.4.Oncogenes, tumor suppressor genes, and cancer (42).

The human body has mechanisms exerted by tumor suppressor genes that regulate cell numbers and ensure that new cells receive DNA that has been precisely replicated(42). Many tumor suppressor gene products act as stop signs to uncontrolled growth and therefore may inhibit the cell cycle, promote differentiation, or trigger apoptosis(43). In contrast to the cellular oncogenes, for which a change in one allele will alter normal function, both alleles of a tumor suppressor gene must lose their function before a tumor develops. The first event is usually a mutation by base exchange or deletion. The second event, affecting the other allele (allele 2), may also be a mutation, but the loss of function more often appears to be from loss of the chromosome after a faulty cell division (mitotic nondisjunction) or other mechanisms. The two-hit hypothesis explains the early onset at multiple sites in the body of an inherited form of cancer like hereditary retinoblastoma(43, 44). The first mutation in a suppressor gene can either be present in the zygote germinal mutation (germ cell mutation due to transmission from an affected parent or due to new mutation) or occur in a single cell of the corresponding tissue (somatic mutation). Loss of function of one allele predisposes the cell to tumor development. With a germinal mutation, all cells are predisposed. Thetumor arises after the loss of function of the second allele. When a somatic mutation occurs in a

single cell, loss of function of both alleles rarely affects the same cell. But with a germ cell mutation, loss of function of the second allele is frequent, since all cells carry the first mutation, and are predisposed. With somatic mutation, the tumor occurs sporadically (is not hereditary) and arises univocally from a single cell clone (43, 44, 45).

2.2.5. Breast Cancer

Breast cancer is a major health problem in all countries and predominantly affects the women population(45). It is the most common type of cancer in women and responses from most of the cancer-related death among women population(45). In 2016, it is estimated that 255,180 new cases of breast cancer and 41,070 breast cancer-related deaths will occur in the United States(45, 46). Also, according to 2014 cancer statistics in Turkey, breast cancer (24.9%) is the most common cancer of women in all age groups (46). Although the etiology of breast cancer is not completely known, it is known to be associated with many risk factors(47). The best-known way to fight against breast cancer is to control the risk factors before the disease develops (47, 48). However, it is very difficult to consider all risk factors in breast cancer as it is in all cancers (48). Women have a higher risk of developing breast cancer than men and many factors increase the risk of breast cancer including advanced age, previous malignant or benign tumors, family history of breast cancer and genetic susceptibility, menstrual history, fertility, breastfeeding, hormonal activity, physical activity, exposure to radiation, alcohol use, fatty diet, and body weight (Figure 2.5 & 2.6) (49) .

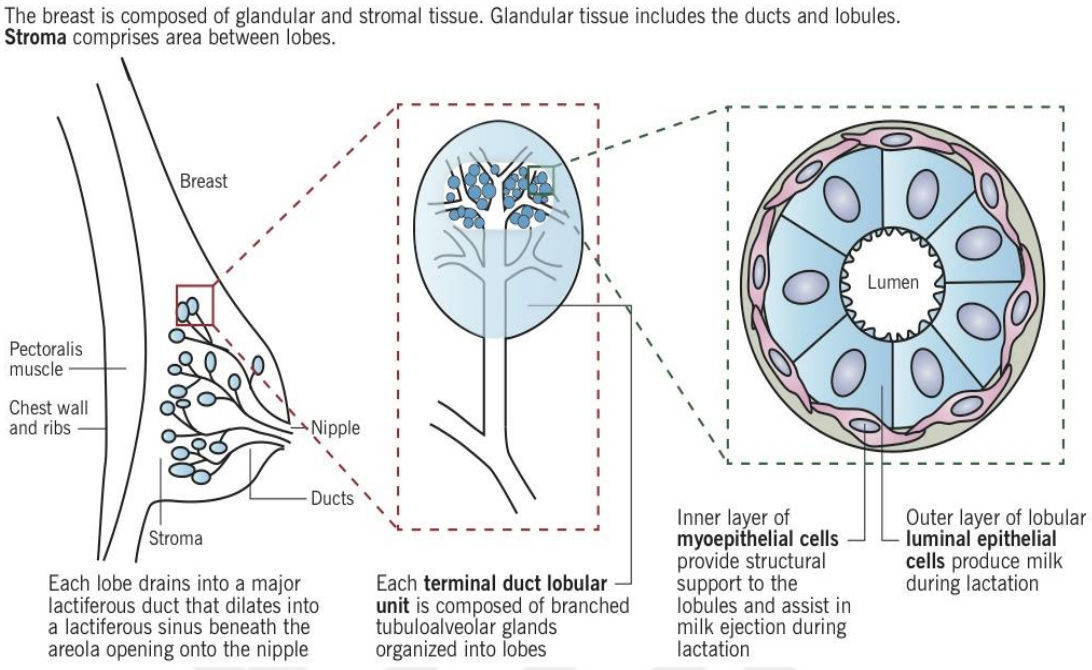


Figure 2.5. Representation of breast anatomy and histology (48).

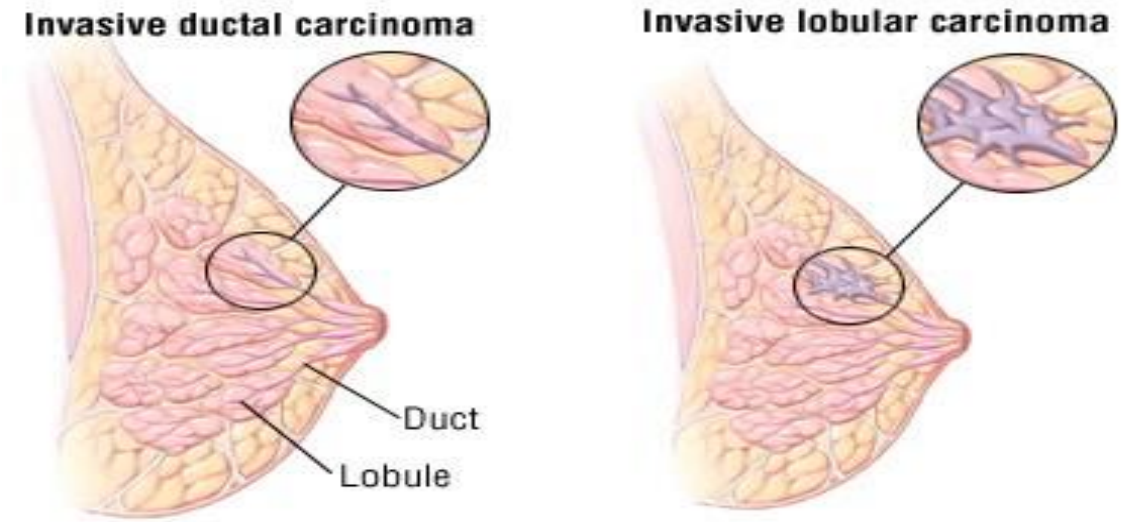


Figure 2.6.Representation of invasive lobular and ductal carcinoma(49).

2.2.6.Pathology and Classification of Breast Cancer

The majority of the malign breast tumors are adenocarcinoma(49). Adenocarcinomas are thought to be originated from the ductal lobular unit. Other malignant tumors such as squamous cell carcinoma, phyllodes tumor, sarcoma, and lymphoma constitute less than 5%(49).

2.2.7. Histologic classification of breast cancer

Histologically, breast carcinomas are divided into two groups as in situ and invasive carcinomas. In situ carcinoma, malign epithelial cells are limited to the basal membrane surrounded by ducts and acinus, whereas in invasive carcinoma, neoplastic cells invade the stroma through the basement membrane (49).Therefore, invasive carcinomas have the capacity to invade lymphatic and blood vessels and metastasize to regional lymph nodes and distant organs(49, 50).Invasive breast carcinomas are tumors that can show morphologically different phenotypic features, and some of them have clinical and prognostic characteristics.Today, the most commonly used method for the histologic classification of breast cancer is the classification recommended by the WHO(50)figure(2.7).

In situ carcinoma

- In situ ductal carcinoma
- In situ lobular carcinoma

Invasive carcinoma

- Invasive ductal carcinoma
- Invasive lobular carcinoma
- Tubular carcinoma
- Invasive cribriform carcinoma
- Medullar carcinoma
- Mucinous carcinoma
- Invasive papillary carcinoma
- Invasive micropapillary carcinoma

- Apocrine carcinoma
- Secretary (juvenile) carcinoma
- Adenoid cystic carcinoma
- Metaplasia carcinoma
- Neuroendocrine carcinoma
- Inflammatory carcinoma

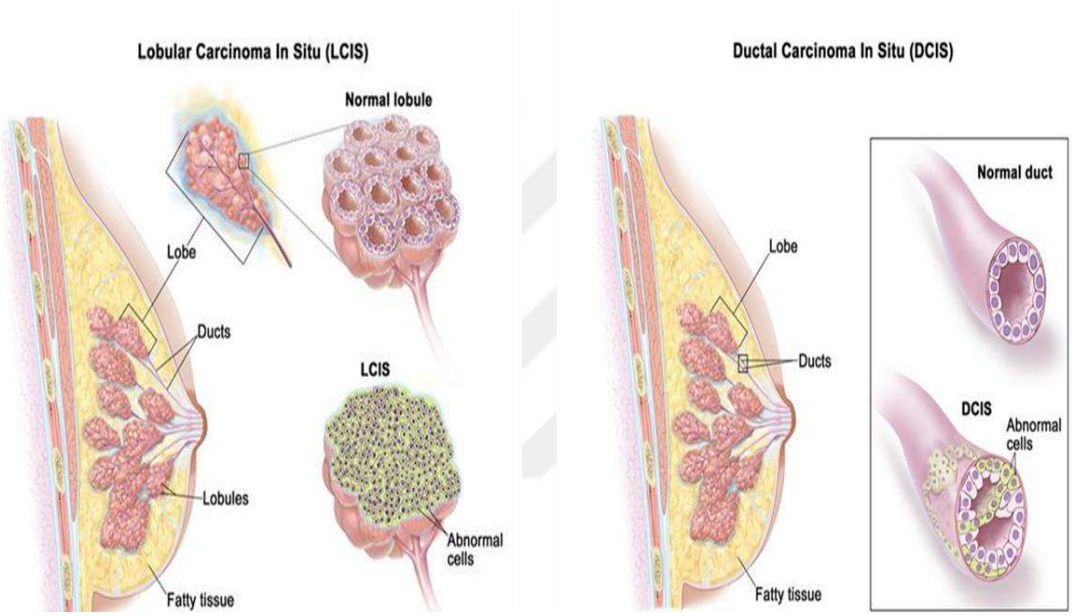


Figure 2.7.Representation of in situ lobular and ductal carcinoma (50).

2.2.8.Lung cancer

Approximately 80% of lung cancers are divided which arepathologicallyand clinically different from non-small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC). Adenocarcinoma (AC) of lung consists of about 50% of all lung cancers. AC is the most common subtype of NSCLC, the group that also comprises large cell carcinoma (LCC) and squamous cell carcinoma (SQCC). Recently, the genomic-based classification of LCC issues this subtype, demonstrating that it can be molecularly sub-classified as either adenocarcinoma or neuroendocrine tumor (51).

Lung cancer accident is gradually rated off in U.S. Men's are continuing to a higher level from women in the U.S. This increase in women in the US is due to the lower level in smoking (52).

Recent epidemiologic and biochemical studies suggest gender differences in susceptibility to tobacco carcinogens. The purpose we conducted an up-to-date, more in-depth evaluation of our earlier observation of a potential gender difference in relative risk (RR) of lung cancer due to smoking(52).

2.2.9. Mechanism of lncRNA in Cancer

Cancer is a genetic diseaseas a result of the dysregulation of the genomic structure. Despite extensive study, the majority of the genetic components of cancer susceptibility have not been linked to individual genes. Exploration of the role of regulatory elements variation, such as ncDNA in gene expression may become a key development in exploring the molecular mechanisms of cancer. Both the small ncRNAs and lncRNAs play a central role in regulating cellular activities in eukaryotes. The alteration and dysregulation of several ncRNAs have been reported in several types of human cancers (52).

The mechanicaleffect of lncRNA on cancer is through which lncRNA contributes to cancer development and is diverse. Evidence suggested that one of the major roles of lncRNA was to guide the site-specificity of chromatin-modifying complexes to affect epigenetic changes. lncRNA could regulate gene expression at the transcriptional and post-transcriptional level by targeting either local or distant genes. Recently, lncRNAs have also shown their tumorigenic potential by modulating transcription of p53 (52, 53).

Dysregulated expression of lncRNA in cancer marks the spectrum of disease progression and may serve as an independent predictor for patient outcomes. Conducted lncRNA and mRNA profile comparison between glioblastoma and normal brain tissue(53).

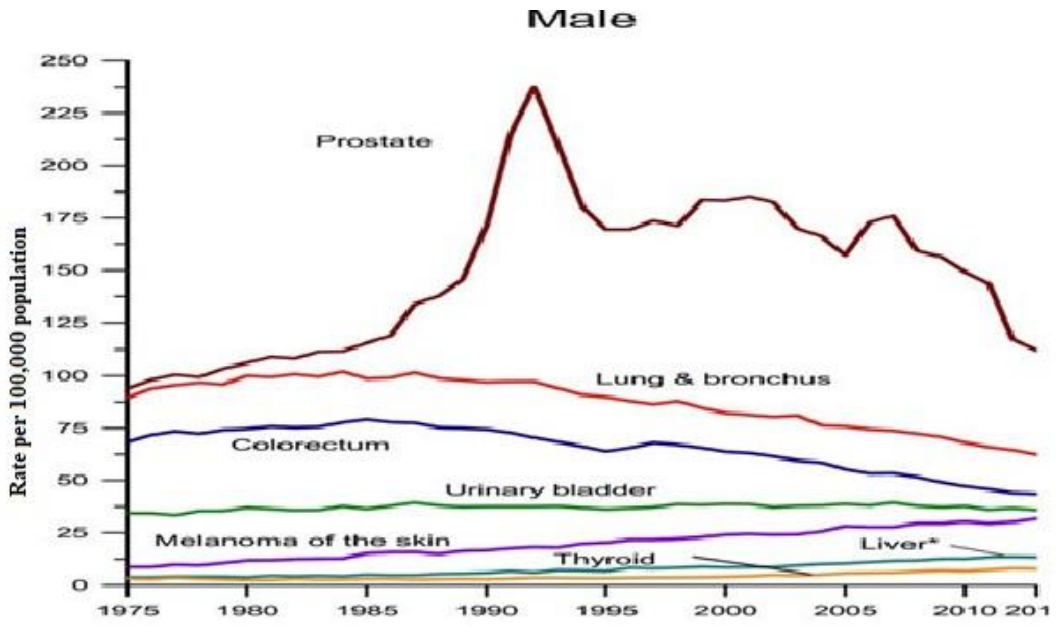


Figure 2.8. Yearly diagnosis in different types of cancer in males rates, United States, 1975-2011, (36).

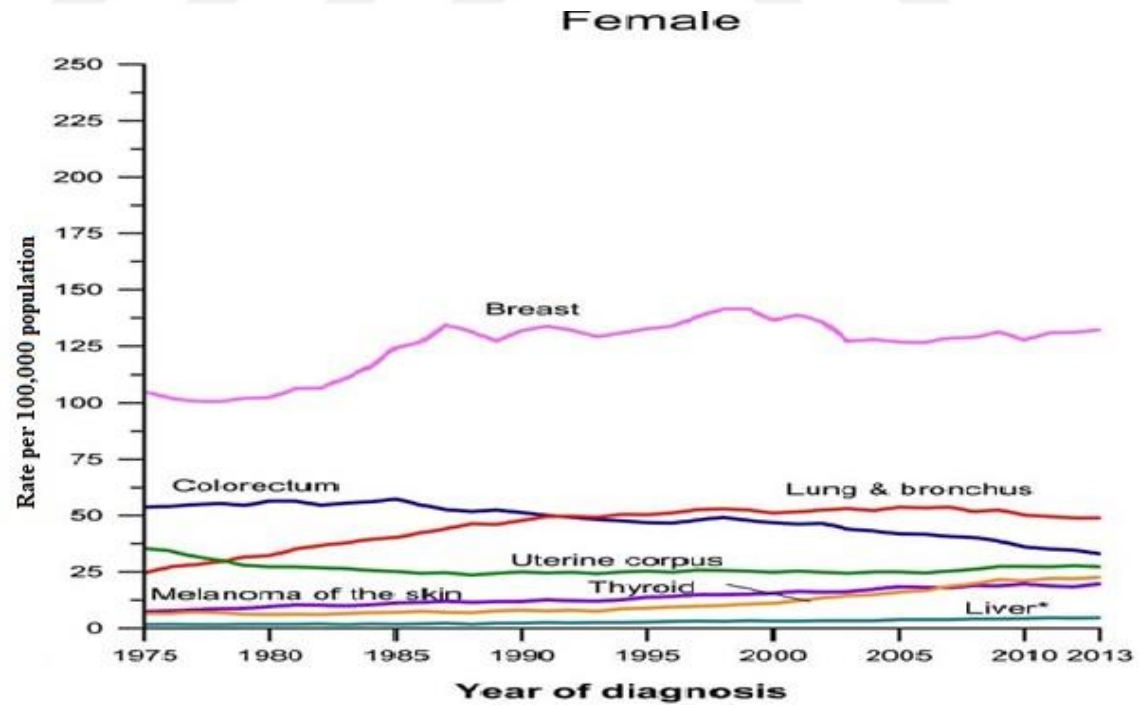


Figure 2.9. Yearly diagnosis in different types of cancer in female rate, United States, 1975-2011, (36).

Table2.3. Turkey in settlements connected with the incidence of cancer showing the number of related cases through 100,000 populations (38).

No.	Province	Rate of cancer	Age
1	Adana	24.1	33.8
2	Ankara	35.1	42.6
3	Edirne	22.6	17.7
4	Erzurm	15.5	21.6
5	Gaziantep	12.4	43.7
6	Istanbul	29.6	25.1
7	Izmir	25.7	28.7
8	Malatya	26.4	29.4
9	Trabzon	27.2	24,4
10	Zonguldak	27.2	35.1

Table2.4. Long noncoding RNAexpressed in theHCC (38).

No.	LncRNA	Dysregulation	Upstream Regulators	Downstream targets	Cellular functions	Clinicopathological Features
1	lncRNA-Dreh	Downregulated	HBxprotein	Vimentin	Cytoskeleton structure	Prognosis
2	lncRNA HEIH	Upregulated		PRC2	Cell cycle	Prognosis
3	lncRNAMVIH	Upregulated		PGK1	Microvessel growth	Metastasis
4	HULC	Upregulated	CREB	miR-372	Proliferation	Prognosis
5	HOTAIR	Upregulated	Suz-Twelve	PRC2	Chrome state	Metastasis
6	MDIG	Upregulated	c-Myc-RB	H3K9me3	DNA repair	Metastasis
7	MALAT1	Upregulated	TGF-beta	Caspase-3	Proliferation	Metastasis
8	MEG3	Downregulated	cAMP	p53	Apoptosis	Prognosis
9	ANRIL	Downregulated		PRC2	tumor suppressor	Metastasis
10	H19	Upregulated		PRC2	tumor suppressor	Metastasis

Table 2.5.Examples of long non-coding RNAs (lncRNAs) associated with human disorders (55).

lncRNA	Cancer/disease	Mechanisms of action
ANRIL	Neurofibromatosis type 1, prostate cancer	Chromatin modification via the recruitment of the Polycomb Repressive Complex 2 (PRC2) at the INKB/ARF/INK4A tumor suppressor locus
HOTAIR	Hepatocellularcarcinoma, colorectal cancer	ChromatinmodificationviatherecruitmentofPRC2 and LSD1in trans.
H19	Colorectal, gastric, breast, lung, esophageal	Chromatin modification via the recruitment of PRC2; Decoy for miR-Let-7;
MALAT1	Lung cancer	Alternative splicing regulation
MIAT	Schizophrenia	Component of the nuclear matrix involved in mRNA splicing
PCAT-1	Prostate cancer	Chromatin modification via the recruitment of PRC2
BACE1-AS	Alzheimer's disease	increase in mRNA stability
GAS5	Breast, bladder cancers	Decoy for the glucocorticoid receptor
PTENP1	Prostate cancer	miR decoy
KCNA2-AS	Neuropathic pain	Decrease of KCNA2 expression

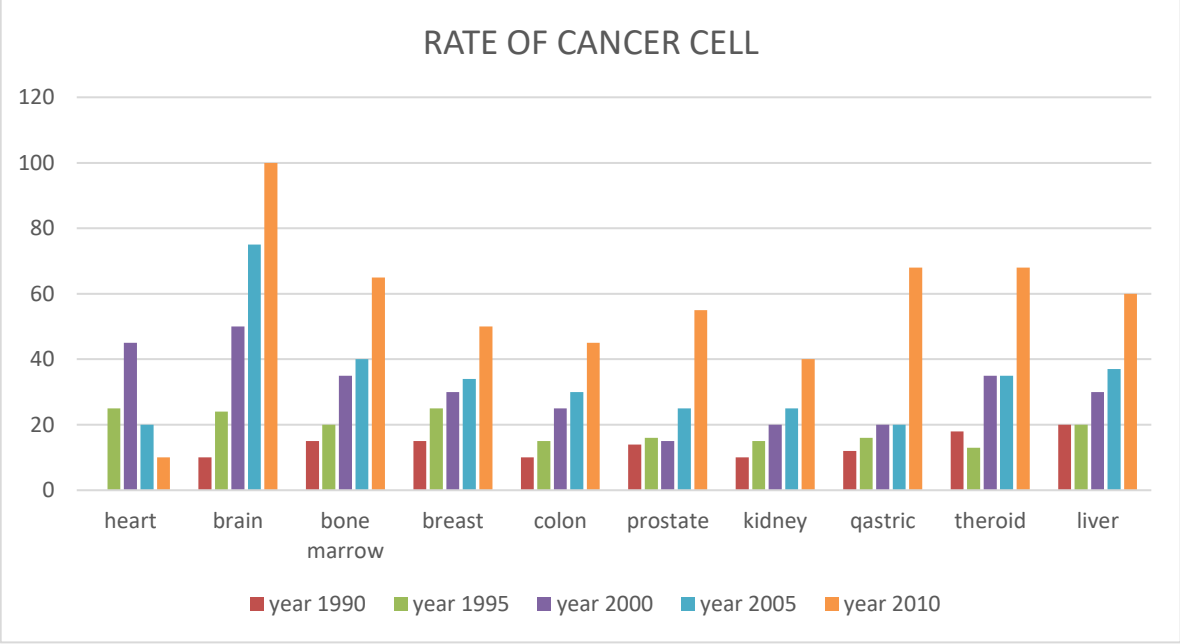


Figure2.10. Year-round elevation of the cancer cell(70).

2.3.1. Natural antisense transcripts (NATs)

Antisense RNA is a single strand RNA that is complementary to a messenger RNA strand transcribed with a cell they are introduced in a cell to inhibit the translation machinery by base-pairing with the sense RNA and activating the RNAseH to develop a particular novel transgenic.Antisense therapy is a model of treatment for infection or genetic disorder,and switch off the pathogenic gene by activating the degrading enzyme RNAseH.Antisense drugs are being researched to treat cancer HIV,CMV.

Antisense transcripts (NATs), a class of long non-coding RNAs (lncRNAs), act by expression levels enhancing or decreasing by binding to noncoding or protein-encoding RNAs. Antisense transcripts that bind to noncoding RNAs are called lncNATs. In recent years, lncRNAs, miRNAs, and other non-coding RNAs have been identified in a variety of cancer pathways that have functions for the diagnosis, and treatment of cancer (54).

These are endogenous RNA molecules that are partially or completely complementary to protein-encoding transcripts. According to their genomic origins, they are separated from the same genomic region as cis-NAT transcribed from the opposite strand compared to sense transcripts and trans-NAT transcribed from different genomic regions(54, 55). It has been shown that NATs effectively regulate the level of expression of protein-encoding targets, although they are usually expressed at a relatively low level compared to sense transcripts (55). ANRIL is a linkNAT transcribed by the tumor locus INK4A-INK4B gene cluster encoding CDKN2A and CDKN2B (55). NATs also influence gene expression through post-software regulation, such as splicing. During the epithelial-mesenchymal transition, a NAT in the ZEB2 locus is software-activated. This ZEB2 NAT inhibits intron splicing, including an internal ribosome entry site, and positively regulates the ZEB2 protein expression. Regulation of sense transcripts by NATs provides a natural way to increase or decrease protein expression (56).

2.3.2. TSIX

Tsix RNA is an antisense and noncoding RNA repressor of Xist and regulates X chromosome inactivation in humans. Tsix is actually for preventing the inactivation of the X chromosome in extraembryonic lineages where imprinted X-chromosome inactivation (XCI). We show that Tsix has a clear functional important role in extraembryonic development. Tsix can repress Xist, which is silencing by DNA methylation of the Xist promoter. As a consequence of Xist repression reactivation of the inactive X chromosome (Xi) is widely observed (57).

Tsix represses Xist and causes reactive of Xi-linked GFP transgene throughout development. Expression patterns of pairs of sense and antisense transcripts suggest that antisense transcription can activate or repress gene expression. X-chromosome inactivation (XCI) the dosage of X-linked genes between females and males in placental mammals (57). The profile X-chromosome activity in Tsix-mutant-chromosome inactivation results in the stable transcriptional silencing of genes along one of the two X-chromosomes in female mammals Xist RNA is believed to initiate epigenetic silencing of genes in cis by physically coating the X-chromosome from which it is transcribed and freeze proteins that catalyze heterochromatin formation Tsix transcription across the Xist promoter,conversely, is proposed to inhibit Xist expression because of its ability to repress Xist, the Tsix locus is postulated to be the site where molecular signals converge to help ensure that one X-chromosome re-remains active in both males and females(58).Tsix begins 15 kb downstream of Xist and spans 40 kb of sequence. In mammals, dosage compensation is achieved by X inactivationand is regulated in cis by the X-inactivation center (Xic) and Xist. The Xic controls X-chromosome counting, choice of X to inactivate and initiation of silencing. Xic action freeze in a change in Xist RNA property from a scarce, unstable RNA to highly expressed Xist RNA that coats the future inactive X.Deleting a 65-kb region downstream of Xist results in constitutive Xist expression and X inactivation, implying the presence of a cis-regulatory element.Tsix sequence is conserved at the human XIC. Tsix RNA has no conserved Open Reading Fragment (ORFs), is seen exclusively in the nucleus and is localized at Xic. Before the onset of X inactivation, Tsix is expressed from both X chromosomes. At the onset of X inactivation, Tsix expression becomes monoallelic, is associated with the future active X and

persists until Xist is turned off. Tsix has features suggesting a role in regulating the early steps of X inactivation, but not the silencing step (Figure 2.11) (59).

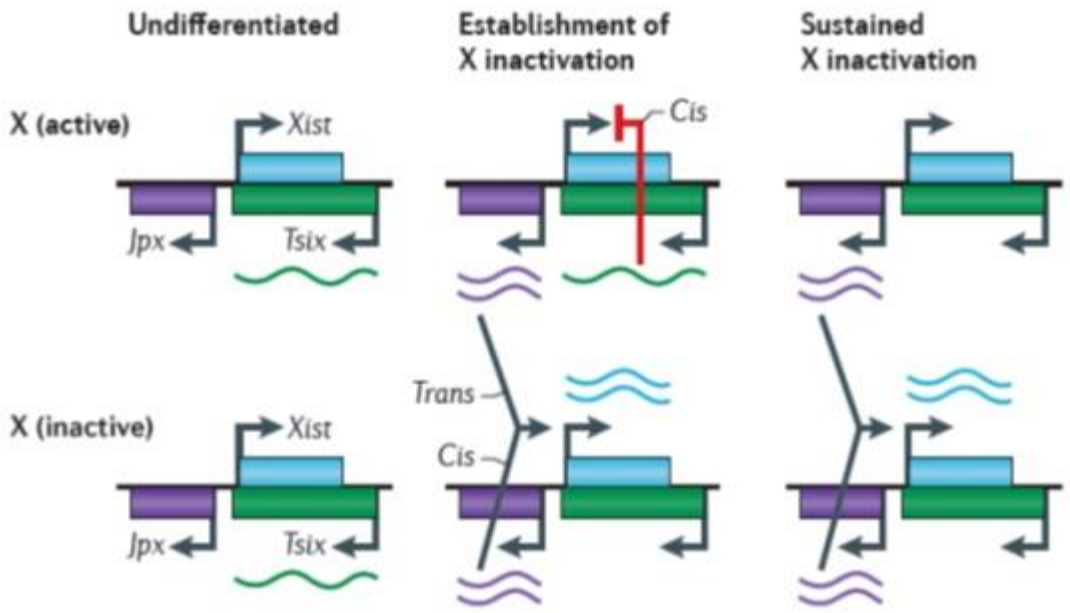


Figure 2.11. The mammalian dosage compensation lncRNA, Xist, is silenced on the active X chromosome in cis by the antisense lncRNA (88).

X inactivation is the mammalian method for X-chromosome dosage compensation, but this developmental process varies among humans. Such types of variations providethe components of the pathway. Tsix noncodes transcript antisense to the murine Xist transcript and is expressed in the mouse embryo of X inactivation, it has been shown to play an important role in imprinted X chromosome inactivation in the mouse placenta. We have identified its inside human Xchromosomeinactivation center (XIC). Human TSIX produces a 130-kb transcript that is expressed only in cells of fetal origin, it is expressed from human XIC transgenes in mouse embryonic stem cells and from human embryoid body-derived cells, but not from human adult somatic cells. Differences in the structure of human and murine genes indicate that human TSIX was truncated during evolution. These differences could explain the fact that X inactivation is not imprinted in human placenta, and they raise questions about the role of TSIX in random X inactivation (60).

The important produce events responsible for an active X in mammalian cells are science yearunknown, but some players have been identified. The key control region on the X chromosome, from which inactivation is initiated and signals are spread, has been mapped to Xq13.2 and is referred to as the X inactivation center (XIC in humans and Xic in mouse). X chromosomes can be programmed toinhibit because of the X inactive specific transcript gene XIST in human and Xist in mouse located within this region.(60). The lncRNA XIST exhibits oncogenic properties via regulation of miR-449a and Bcl-2 in human nonsmall cell lung cancer (NSCLC) (61).

2.3.3. HAGLR

HOXD-AS1 is one of the new lncRNAs in HOXD and plays an important role in the expression of HOXD-AS1. It is associated with the expression of HOXD1 and HOXD3 genes, an indicator of neuroblastoma progression through integrative analysis of the non-coding transcript.The characterized pattern of expression of HOXDAS1in the human body by qRT-PCR analysis of human organ cDNA panel. Theyfound that HOXD-AS1 is expressed in multiple tissues with the highest levels of expression in kidney, colon and testes and the lowest in liver, heart, pancreas, and stomach (62).

HOXD-AS1 showed highly correlated expression with neighboring protein-coding genes HOXD1 and HOXD3, thus it means thatthey are subject to the important regulatory mechanism. Notably, the non-MNA patient tumors HOXD-AS1 expression significantly correlated with HOXD3 differentially expressed genes identified after knockdown of HOXD-AS1 in SH-SY5Y cells (62).

HOXD-AS1 we can know thatit affects gene expression profile in the context of differentiation of SH-SY5Y cells. This purpose they designed two types of siRNA targeting different regions of HOXD-AS1. Both of these siRNAs blocked of HOXD-AS1 transcript in the retinoic acid-induced SH-SY5Y cells compared to targeting siRNA control (62, 63).This is the new example of the progress developed via the analysis of expression of lncRNAs.

A model of human metastatic neuroblastoma, they found that HOXD-AS1 is a subject to morphogenic regulation, is activated by the PI3K/Akt pathway and itself is involved in the

control of RA-induced cell differentiation. The experiments revealed that HOXD-AS1 controls expression levels of clinically significant protein-coding genes involved in angiogenesis and inflammation, the hallmarks of metastatic cancer (63).

LncRNA it has been proven to act as key molecules in cancer development and progression.Degradationof constant lncRNAs is produced in different tumor tissues and cancer cells, they can serve as oncogenes or tumor suppressors. (HOXD cluster antisense RNA 1) Long non-coding RNA HOXD-AS1 has recently been identified to be involved in the development of several cancers including neuroblastoma, adenocarcinomas and breast cancer. However, the role of HOXD-AS1 in bladder cancer remains unknown(64).(HOTAIR) Model of long noncoding RNA HOX transcript antisense RNA regulating expression of HOX genes in trans.LncRNA HOTAIR transcribed from the HOXC cluster of genes chr 2 binds PRC2 complex of polycomb-group of proteins and targets it to the HOXD cluster chr 12 leading to H3K27 methylation and silencing of neighboring HOXD genes (Figure 2.12) (64).

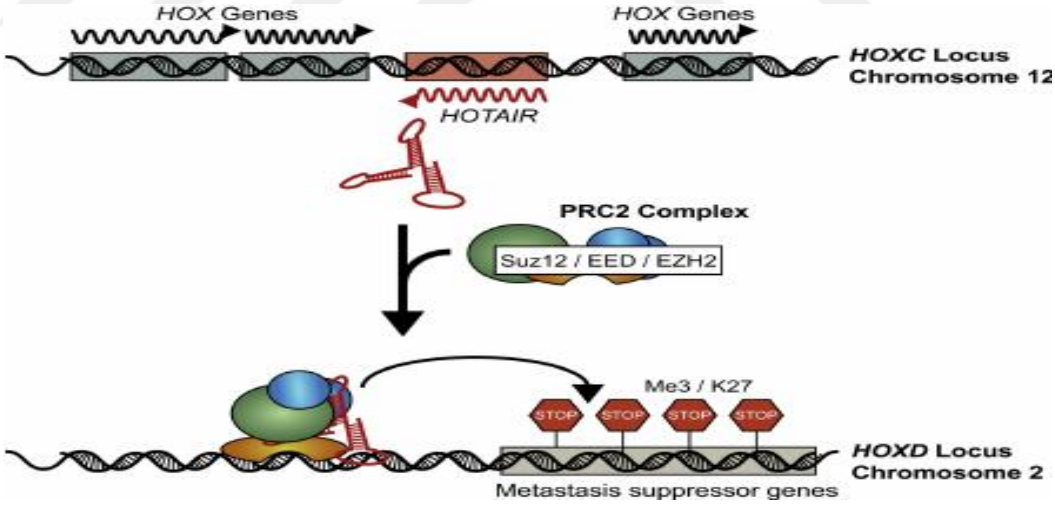


Figure 2.12. Model of long noncoding RNA (lncRNA) HOX transcript antisense RNA (HOTAIR) (15).

2.3.4. LCMT1 antisense RNA

Leucine Carboxyl Methyltransferase-1(LCMT1) is The function of this methylation, required for Normal Progression through Mitosis in Mammalian Cells.LCMT1, the terminal carboxyl group of human protein phosphatase 2A (PP2A) leucine 309 residues methylatesPP2A,a key regulator of many cellular processes, has recently generated additional interest as a potential cancertherapeutic target. The status of PP2A methylation impacts upon the selection of the regulatory subunit by the PP2A core enzyme (65).

Protein phosphatase 2A (PP2A) is a multifunctional phosphatase that plays important roles in many cellular processes including regulation of cell cycle and apoptosis. Because PP2A is involved in so many biological processes, it is highly regulated expression level by both non-covalent and covalent mechanisms that are still being defined. In this study, we havefor PP2A methylation and cell function to the realization of the interest of LCMT-1(65).

PP2A, a multifunctional threonineserine phosphatase, has beencantilevered in the arrangement of cell growth and proliferation, cause to the growth of human cancers primarily at the G2/M transition, (65, 66). PP2A has a different function As structural, B-type regulatory/targeting, and C catalytic. Binding of variant B-type subunits to the core PP2A heterodimer (A/C) alters its substrate(66).

Recently generated additional important as anability to cancer-therapeutic target. PP2A is an important regulator of many cellular processes, including cell-cycle regulation, DNA replication,mRNA translation, signal transduction,and apoptosis,Abnormal activity of PP2A leads to hyperphosphorylation of the tau protein, addition to the disease of Alzheimer's disease. Also, PP2A has been identified as a tumor suppressor in lung and colon cancer, highlighting this molecule as a potential target for novel cancer-therapeutic strategies.PP2A is regulated through two main mechanisms phosphorylation and methylation. Both modifications occur at the carboxyl-terminal motif (residues 294–309) of PP2A, which is highly conserved in all other serine-threonine phosphatases. PP2Ais phosphorylated by the epidermal growth factor (EGF) and insulin receptors and by other tyrosine kinases including

the leukocyte-specific protein tyrosine kinase (LCK) and the viral sarcoma (v-SRC) kinase (67).

2.4.1. Diet and Environmental Factors

Smoking and alcohol and lifestyle, age, radiation, pesticides and otherenvironmental carcinogens, infectious diseases such as lung cancer formations. Also, in many studies, with nutrients and supplement calcium (Ca + 2) uptake also promotes growth and metastasis of lung cancer cellshas been shown to increase lung cancer risk. However, in different studiescalcium has an adverse effect on advanced prostate cancer cases; only low levelsin cancer cases were positively associated with cancer development(68). Highly cooked ethyne 2-amino-1-methyl-6-phenylimidazo (4,5-b) pyridine (PhIP).Studies have shown that excess meat mainly processed and overcooked meat is consumed by the lungcancer risk. Also, total fat and animal fatsincrease correlated positively with cancer risk (68).

Soy proteins, tomatoes, and lycopene, vitamin E, selenium, fish oil and ω-3fatty acids, crucifer and green tea metabolism in prostate cancer, cell-cycle, mitochondrial membrane potential conservation, insulin-like growthfactor (IGF) an activity to carcinogenic pathways such as Akt signaling and oxidative stress responsethey show chemopreventive properties. For example, at higher levels in domesticationlycopene A549, lung cancer, a lipophilic carotenoid foundby decreasing the level of cyclin D, cyclin E and cyclin-dependent kinases in cell linescausing venous insufficiency (69).

3.MATERIALS and METHODS

3.1. Collection of cell lines

In this study, 1 ml of cell line samples were collected in order to isolate RNAs from 7different cell lines. Samples were stored at -80 °C in Eppendorf tubes to be used for RNAisolation.

Table 3.1. Information of 7 cell line patients

No.	NAME OF CELL	Name of tissue	Biosafety Level	Organism	Cell Type	nationality
1	A549	Lung cancer	1	*Homo sapiens*	Tumor	U.S
2	CRL2329	Breast cancer	1	*Homo sapiens*	Lobular	U.S
3	CRL4010	Breast normal	1	*Homo sapiens*	epithelial	U.S
4	MDAMB	breast cancer	1	*Homo sapiens*	Ductal	U.S
5	Beas2B	Normal lung	1	*Homo sapiens*	epithelial	U.S
6	CRL8798	Normal breast	1	*Homo sapiens*	lobular	U.S
7	MCF7	Breast cancer	1	*Homo sapiens*	Tumor	U.S

3.2. Cell Culture

All cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum100 U/mL penicillin, and 100 mg/mL streptomycin in a humidified atmosphere containing 5% CO2 at 37 °C, at Medical Biology Department Cell Culture Lab., Gaziantep University.

3.3.Primer Design

For this study, appropriate synthetic primers were designed for intron regions of *Tsix,HAGLR, LCMT1AS, and NAV2AS5*genes by using the NCBIPrimer Blast database. Primer sequences, length, annealing temperature, GC content and PCR product length of primers designed for intronsequences of *HAGLR, LCMT1AS, and NAV2AS5*genes were shown in (Table 3.2).

Table 3.2.Primer design

	sequence (5'->3')	Primer lenght	tempertur e (°C)	GC%	PCR product(bp)
TSIX	GTTGCATCAGCTGTCCTCCT	17	57.75	64.71	221
	AAAAAGGGGTTGGGGTAGG	20	61.90	60.00	
HAGLR	ACCAGACCTACTCTTCCGCT	20	59.88	55.00	246
	GGGAAGAGCCAAGTCAGAC	20	60.03	55.00	
LCMT1AS	ATCTGGTGAGCCAGGTAGGA	20	59.53	55.00	207
	GGGAAGAGCCAAGTCAGAC	21	59.44	52.38	
NOV2AS	CCCACTGTGAGAACCCCTTC	20	59.96	60.00	209
	GAGACCCATGCCAGTGTGTG	20	60.96	60.00	

3.4. RNA Isolation from cell line :

After cells reach 80-90% density, they are removed for RNA isolation under appropriate conditions. RNA was extracted from cell culture using High Pure RNA Isolation (Roche, Mannheim, Germany) kit. The RNA isolation protocol is as follows;

1- The cells with appropriate density are removed with Trypsin, and DMEM containing FCS is added to stop the effect of trypsin.

2- Cells are centrifuged at 3500 rpm for 5 min. The supernatant is removed without touching the pellet. The remaining pellet is resuspended in 200 µl PBS.

3- Add 400 µl of Lysis Buffer to this mixture and vortex for 15 seconds.

4- The whole mixture is transferred to filter tubes, centrifuged for 30 seconds at 9200 rpm. The lower part is discarded.

5- Add 100 µl (10 µl DNAse and 90 µl DNAse incubation buffer) to the filtered tubes and wait at room temperature for 45 minutes.

6- Add 500 µl Wash Buffer I and centrifuge at 9200 rpm for 30 seconds. The lower part is discarded.

7- Add 500 µl Wash Buffer II and centrifuge at 9200 rpm for 30 seconds. The bottom tube is replaced with the new one.

8- Add 200 µl of Wash Buffer II and centrifuge for 2 minutes at 11800 rpm. The lower tube is discarded and a new tube is inserted.

Add 50 µl of Elution Buffer and wait for 1 minute at room temperature. Centrifuge at 9200 rpm for 1 minute. The filtered tube is discarded.

9- Measurements are made on the NanoDrop 1000 to determine the amount of RNA.

10- RNAs are stored at -80 ºC until the working period.

3.5. RNA Quantitation

Determination of quantity and quality of obtained RNA samples was done by detecting the A260/A280 ratio usingNanodrop spectrophotometer. For PCR reactions, RNA was diluted according to their density (Table 3.3).

Table 3.3.Concentrations of RNA

.

No	Name of cell	ng/µl	260/280
1	MCF7	1502	2,07
2	MDA-MD	1451	2,05
3	CRL2327	761	2,06
4	CRL4010	336	2,02
5	A549	329	2,07
6	Beas2B	814	2,05
7	CRL8798	1060	2.03

3.6.cDNA components and their amounts

Single-stranded cDNA synthesis from RNA-isolated samples and tissue RNAs was performed with Maxima H Minus First Strand cDNA Synthesis Kit # K1652 (Thermo Scientific) (Table 3.4). From the cell culture samples, cDNA synthesis was performed by adding RNA at a final concentration of 2 µg / µl RNA and tissue RNA at a final concentration of 1 µg / µl RNA.

Table 3.4.cDNA components and their amounts.

cDNA components	amounts(µl)
RNA sample	10 µl
Random primer	1 µl
dNTP	1 µl
Distilled water	3 µl
RT Buffer	4 µl
H minus Enzyme	1 µl
Total volume	20 µl

3.7. Real-Time PCR (qPCR) Analyzes

qPCR was performed to determine expression levels of target genes more effectively in tissues and cell lines. For this experiment, a Rotor-Gene Q (QIAGEN,) Real-Time PCR instrument was used. qPCR experiments were performed in the direction of the manufacturer's firm using Maxima SYBR Green / ROX qPCR Master Mix (# K0251). Table (3.5) shows the components and amounts to be included in each tube for qPCR analysis.

Table 3.5. RT PCR components and their amounts for the intronic region of TSIXgene

PCR components	amounts(µl)
Master mix	7.5
10 pm Forward primer	0.5
10 pm reverse primer	0.5
Distilled water	5
Template cDNA	1.5
Total volume	15

Table 3.6. RT PCR components and their amounts for the exonic region of HAGLRgene

PCR components	amounts(µl)
Master mix	7.5
10 pm Forward primer	0.5
10 pm reverse primer	0.5
Distilled water	5
Template cDNA	1.5
Total volume	15

Table 3.7. RT PCR components and their amounts for the intronic region of NOV2ASgene

PCR components	amounts(µl)
Master mix	7.5
10 pm Forward primer	0.5
10 pm reverse primer	0.5
Distilled water	5
Template cDNA	1.5
Total volume	15

Table 3.8. RT PCR components and their amounts for the intronic region of LCMTAS2gene

PCR components	**amounts(µl)**
Master mix	7.5
10 pm Forward primer	0.5
10 pm reverse primer	0.5
Distilled water	5
Template cDNA	1.5
Total volume	15

Table 3.9.RT PCR conditions of *TSIX* gene intron regions

	Step	**Temperature (°C)**	**Duration**
1	1st denaturation	95	5 min
2	2nd denaturation	95	45 sec
3	Primer annealing	*	45 sec
4	Extention	72	45 sec
5	Last extention	72	5 min
		4	4 min
2 to 4	40 cycles		

Table 3.10.RTPCR conditions of *HAGLR* gene intron regions

	Step	Temperature (°C)	Duration
1	1st denaturation	95	5 min
2	2nd denaturation	95	40 sec
3	Primer annealing	*	45 sec
4	Extention	72	40 sec
5	Last extention	72	7 min
		4	4 min
2 to 4	35 cycles		

Table 3.11. PCR conditions of *LCMT1AS* gene intron regions

	Step	Temperature (°C)	Duration
1	1st denaturation	95	7 min
2	2nd denaturation	95	45 sec
3	Primer annealing	*	40 sec
4	Extention	72	45 sec
5	Last extention	72	5 min
		4	4 min
2 to 4	35 cycles		

Table 3.12. PCR conditions of NOV2AS gene intron regions

	Step	Temperature (°C)	Duration
1	1st denaturation	95	5 min
2	2nd denaturation	95	40 sec
3	Primer annealing	*	45 sec
4	Extention	72	40 sec
5	Last extention	72	7 min
		4	4 min
2 to 4	35 cycles		

3.8. RT-PCR conditions of GAPDH

Housekeeping genes are defined as genes with constant expression levels in all cells of an organism under normal and pathophysiological conditions. In this regard, the spike genes are used for normalization in gene expression assays. In PCR analyses of the expression level of TSIX,HAGLR,NOV2AS1, and LCMT1 gene in different cell lines, the GAPDH gene was used as the haplotype gene. Appropriate temperature conditions and PCR components for amplification of the GAPDH gene are shown in (Table 3.13& 3.14).

Table 3.13. **GAPDH'ın RT-PCR componentand amount**

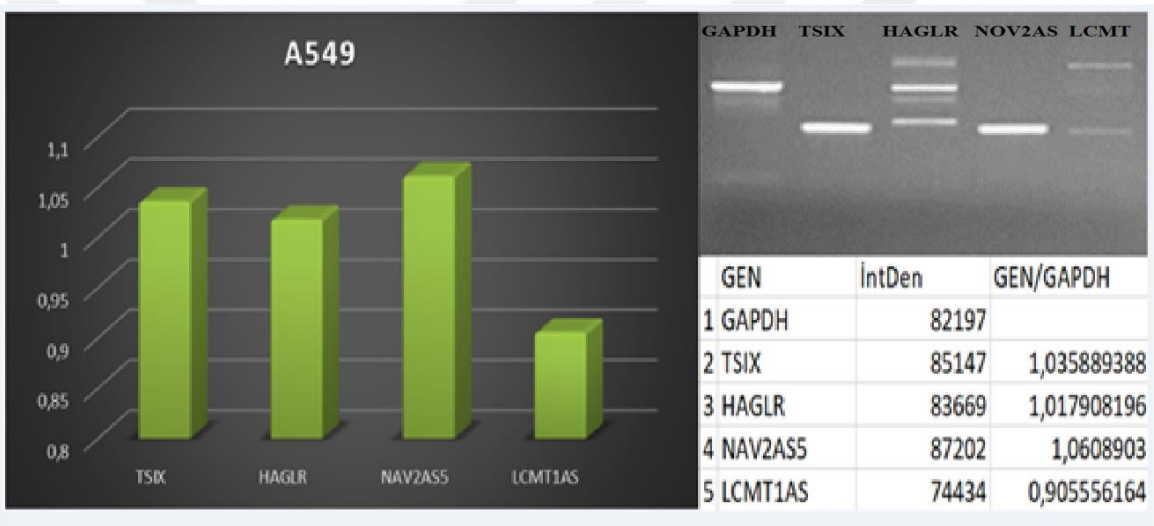
Component	amount (µl)
Distel water	14,875
10X PCR	2,5
25 mM $MgCl_2$	2
2 mM dNTPs	2,5
forward primer (F) 10 pM	1
reverse primer (R) 10 pM	1
5 U/µl Taq polemerase	0,125
cDNA	1
Total	**25**

Table 3.14. PCR conditions of GAPDH'ın RT-PCR

	Temperture (ºC)	time (min)	Replication
1st denaturation	95	05:00	1
2nd denaturation	95	00:30	
annealing	62	00:30	30
Polimerazation	72	00:30	
Extention	72	5:00	1
	+4	4 min	

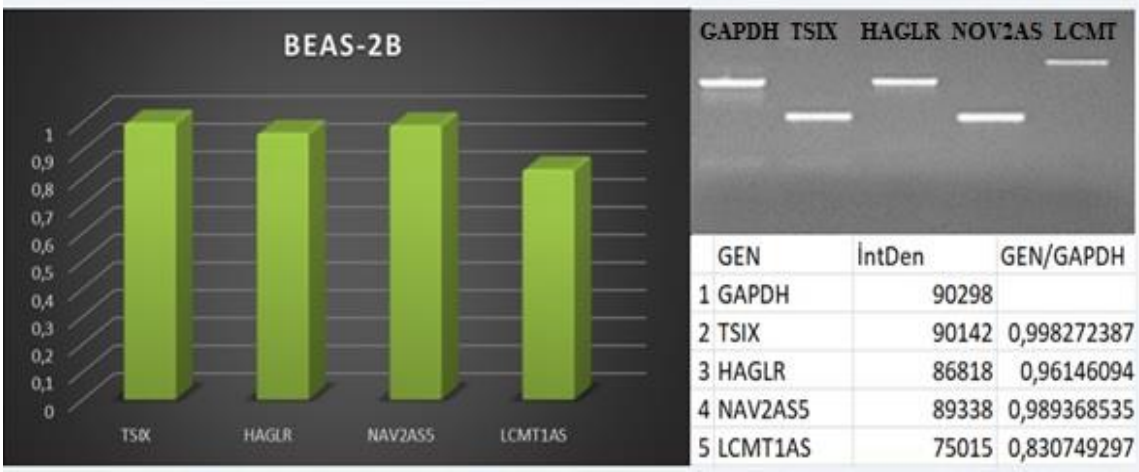
4.Results

4.1. Gene expression results of TSIX,HAGLR,NOV2AS5, and LCMT1ASin different cell lines. The expression level of TSIX,HAGLR,NOV2AS5, and LCMT1AS has been analyzed in 7 different human cell lines. Gene expression analyses were performed both by partial-quantitative PCR and by Real-Time PCR methods. TSIX has been shown to be expressed mostly in lung and breast tissues. TSIX was found to show expression in lung cancer cell lines. was measured by the ImageJ software program (version 1.46r, downloaded from http://imagej.nih.gov/ij) (Figure4.1& 4.2).



	GEN	İntDen	GEN/GAPDH
1	GAPDH	82197	
2	TSIX	85147	1,035889388
3	HAGLR	83669	1,017908196
4	NAV2AS5	87202	1,0608903
5	LCMT1AS	74434	0,905556164

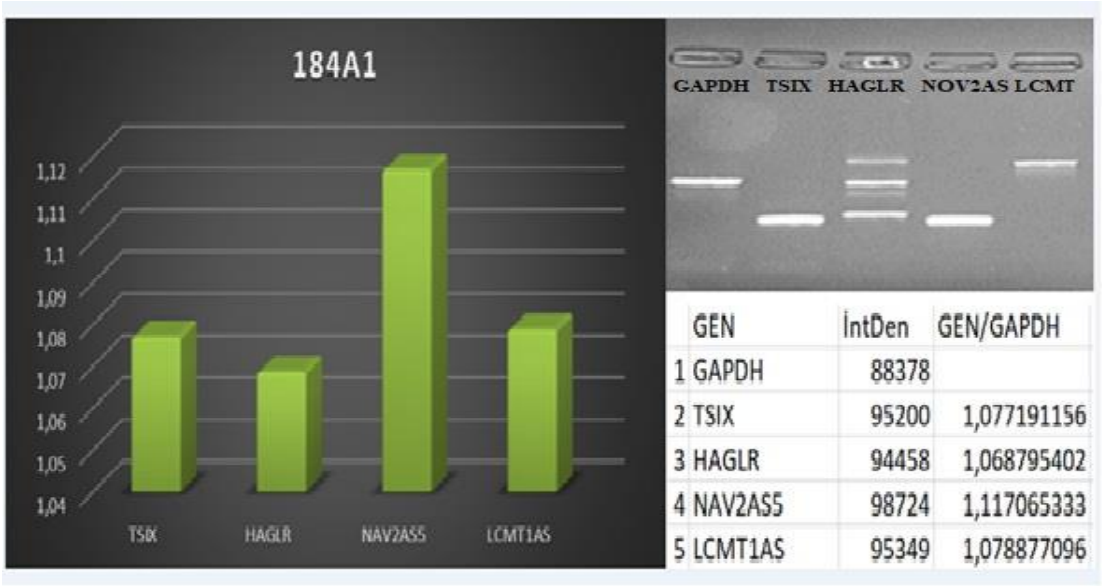
Figure 4.1. Expression levels of TSIX,HAGLR,NOV2AS5,LCMT1AS, and GAPDH genes by RT-PCR



	GEN	İntDen	GEN/GAPDH
1	GAPDH	90298	
2	TSIX	90142	0,998272387
3	HAGLR	86818	0,96146094
4	NAV2AS5	89338	0,989368535
5	LCMT1AS	75015	0,830749297

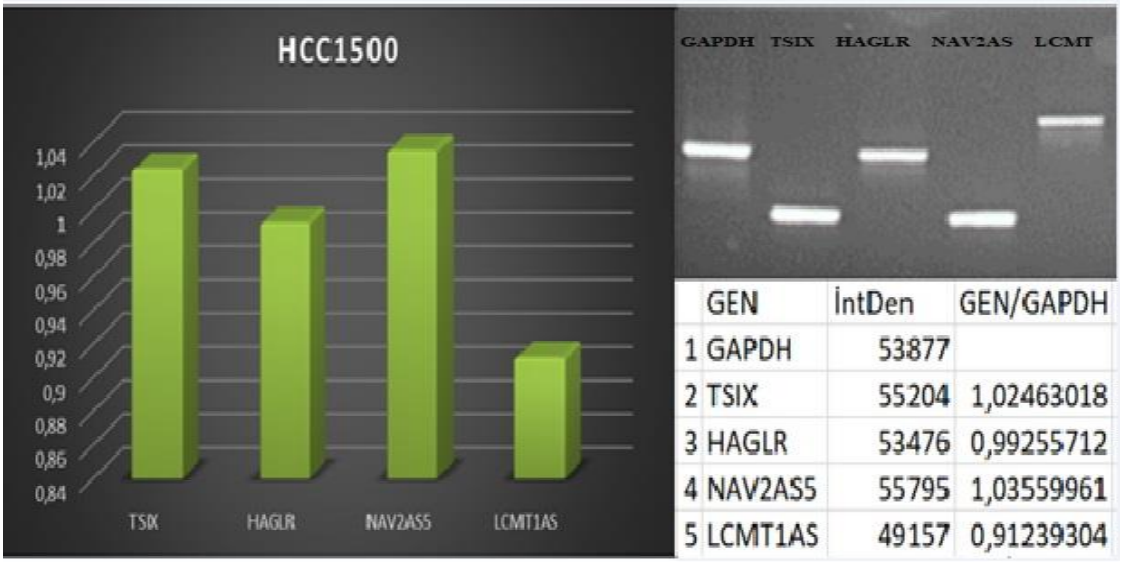
Figure 4.2. Expression levels of TSIX,HAGLR,NOV2AS5,LCMT1AS, and GAPDH genes by RT-PCR

4.2. Gene expression results of TSIX,HAGLR,NOV2AS5, and LCMT1AS in different cell lines. The expression level of TSIX,HAGLR,NOV2AS5, and LCMT1AS has been analyzed in 7 different human cell lines. Gene expression analyses were performed by Real-Time PCR methods. TSIX has been shown to be expressed mostly in lung and breast tissues. TSIX was found to show expression in the breast cancer cell line(Figure 4.3, 4.4, 4.5, and 4.6).



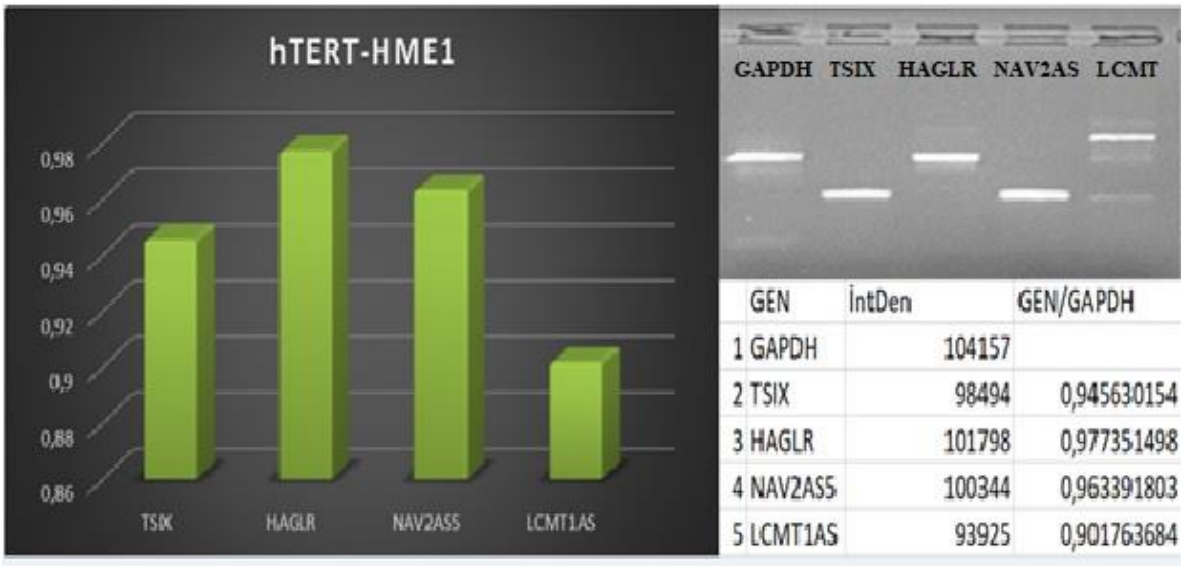
	GEN	İntDen	GEN/GAPDH
1	GAPDH	88378	
2	TSIX	95200	1,077191156
3	HAGLR	94458	1,068795402
4	NAV2AS5	98724	1,117065333
5	LCMT1AS	95349	1,078877096

Figure 4.3. Expression levels of TSIX,HAGLR,NOV2AS5,LCMT1AS, and GAPDH genes by RT-PCR.in breast cancer cell line



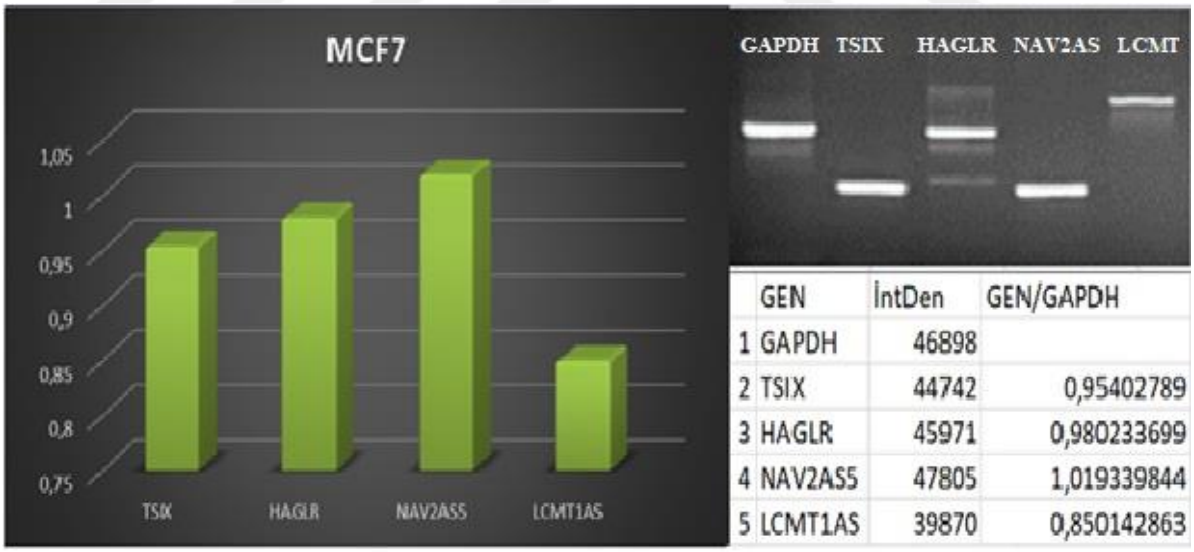
	GEN	İntDen	GEN/GAPDH
1	GAPDH	53877	
2	TSIX	55204	1,02463018
3	HAGLR	53476	0,99255712
4	NAV2AS5	55795	1,03559961
5	LCMT1AS	49157	0,91239304

Figure 4.4. Expression levels of TSIX, HAGLR,NOV2AS5,LCMT1AS, and GAPDH genes by RT-PCR.in breast cancer cell line HCC1500



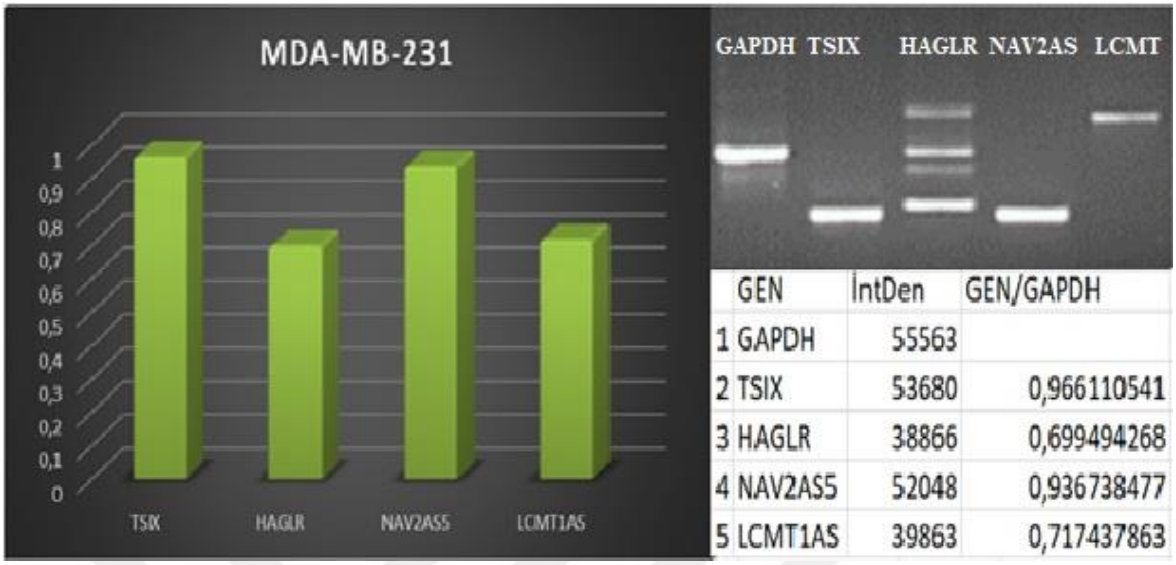
	GEN	İntDen	GEN/GAPDH
1	GAPDH	104157	
2	TSIX	98494	0,945630154
3	HAGLR	101798	0,977351498
4	NAV2AS5	100344	0,963391803
5	LCMT1AS	93925	0,901763684

Figure 4.5.Expression levels of TSIX,HAGLR,NOV2AS5, LCMT1AS, and GAPDH genes by RT-PCR in normal cell line Htert-HME1



	GEN	İntDen	GEN/GAPDH
1	GAPDH	46898	
2	TSIX	44742	0,95402789
3	HAGLR	45971	0,980233699
4	NAV2AS5	47805	1,019339844
5	LCMT1AS	39870	0,850142863

Figure 4.6. Expression levels of TSIX,HAGLR,NOV2AS5,LCMT1AS, and GAPDH genes by RT-PCR in breast Cancer cell line



	GEN	İntDen	GEN/GAPDH
1	GAPDH	55563	
2	TSIX	53680	0,966110541
3	HAGLR	38866	0,699494268
4	NAV2AS5	52048	0,936738477
5	LCMT1AS	39863	0,717437863

Figure 4.7. Expression levels of TSIX,HAGLR,NOV2AS5,LCMT1AS, and GAPDH genes by RT-PCR in breast Cancer cell line,MDA-MB-231.

4.3. The expression level of HAGLR in lung

The expression level of HAGLR is shown in 2 different cell lines. Gene expression analyses were performed by Real-Time PCR methods (Figure 4.8). HAGLR has been shown to have the highest expression level in A549 cells following expression in the A549 cell line, the most among cell lines. HAGLR was found to be the lowest in BEAS-2B, control group (Table 4.1).

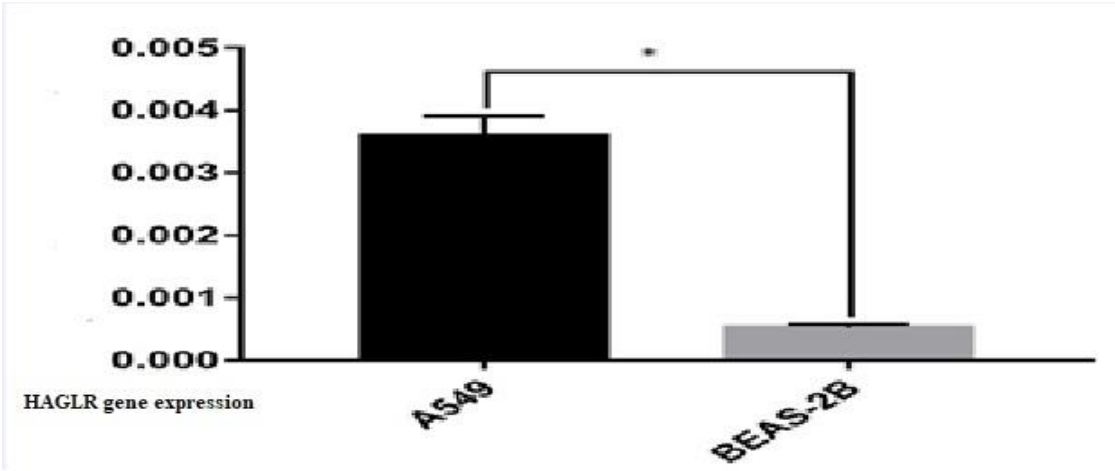


Figure 4.8. HAGLR has been shown to be expressed mostly in lung cancer, and the control groupwas found to show the expression of cancer and normal cell line.

Table 4.1. Statistical analysis of the HAGLR gene in lung.

P value	0.0383
P value summary	*
Significantly different (P < 0.05)?	Yes

4.4. The expression level of HAGLR in breast

The expression level of HAGLR is shown in 5 different cell lines. Gene expression analyses were performed by Real-Time PCR methods (Figure 4.9). HAGLR has been shown to have the highest expression level in CRL2329 cells for Breast cancerfollowing expression in the MCF7,MDA-MB-231,CRL2329, and CRL4010 cell line, the most among cell lines. HAGLR was found to be the lowest in MCF7,MDA-MB-231,CRL2329,CRL4010, control group, and cancer group (Table 4.2).

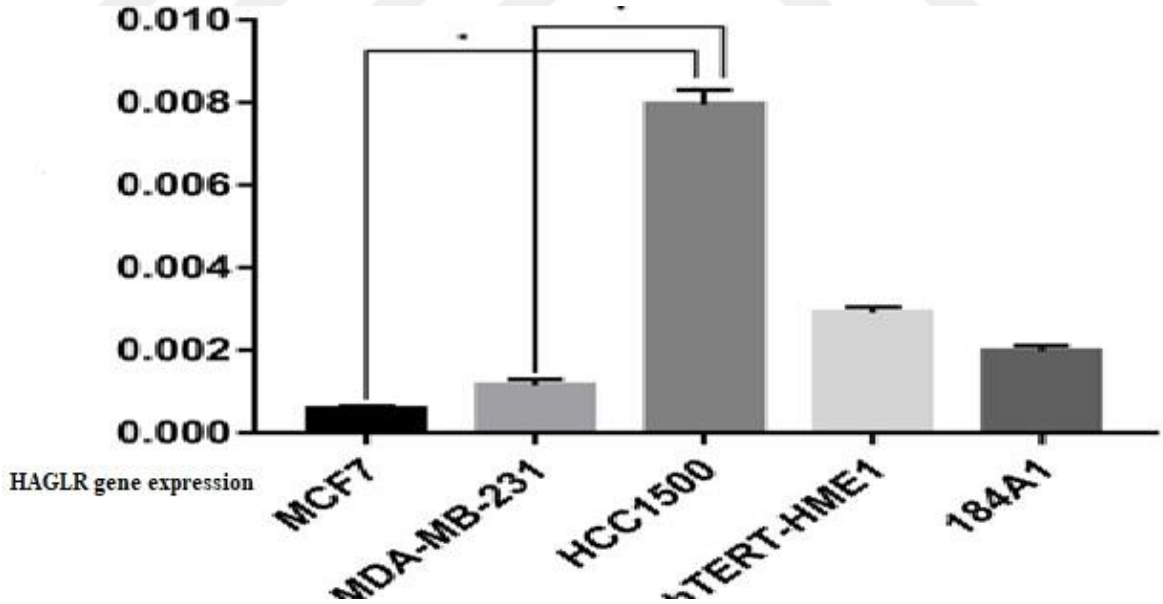


Figure 4.9. HAGLR has been shown to be expressed mostly in breast cancer, and the control group was found to show the expression of cancer and normal cell line.

Table 4.2. Statistical analysis of HAGLR gene in breast

MCF7 vs. HCC1500	-0.007756	-0.01474 to -0.0007686	Yes	*	0.0451
MCF7 vs. hTERT-HME1	-0.002109	-0.0136 to 0.009384	No	ns	0.2476
MCF7 vs. 184A1	-0.001479	-0.01217 to 0.009214	No	ns	0.3162
MDA-MB-231 vs. HCC1500	-0.007225	-0.01248 to -0.001966	Yes	*	0.0366
MDA-MB-231 vs. hTERT-HME1	-0.001577	-0.0148 to 0.01165	No	ns	0.3575
MDA-MB-231 vs. 184A1	-0.0009468	-0.00991 to 0.008017	No	ns	0.3943

4.5. The expression level of TSIXin lung

The expression level of TSIX is shown in 2 different cell lines. Gene expression analyses were performed by Real-Time PCR methods (Figure 4.10). TSIX has been shown to have the highest expression level in A549 cells following expression in the A549 cell line, the most among cell lines. TSIX was found to be the lowest in BEAS-2B, control group (Table 4.3).

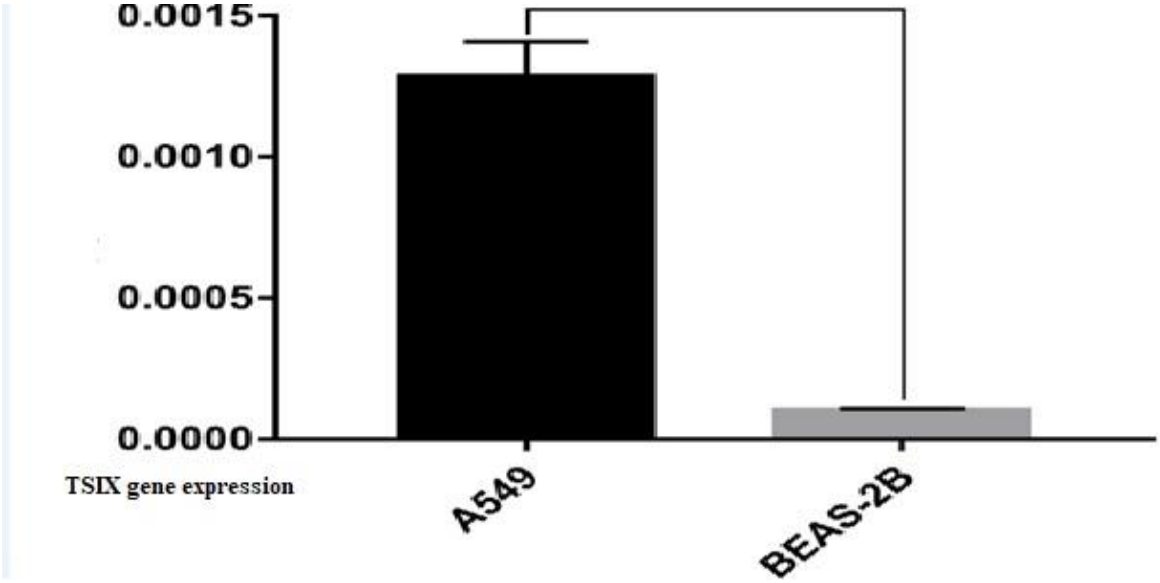


Figure 4.10.TSIX has been shown to be expressed mostly in lung cancer, and the control group was found to show the expression of cancer and normal cell line.

Table 4.3. Statistical analysis of TSIX gene in lung

P value	0.0443
P value summary	*
Significantly different (P < 0.05)?	Yes

4.6. The expression level of TSIX in breast

The expression level of TSIX is shown in 5 different cell lines. Gene expression analyses were performed by Real-Time PCR methods (Figure 4.11). HAGLR has been shown to have the highest expression level in CRL2329 cells for besat cancer following expression in the MCF7,MDA-MB-231,CRL2329,CRL4010 cell line, the most among cell lines. HAGLR was found to be the lowest in MCF7,MDA-MB-231,CRL2329,CRL4010, control group, and cancer group (Table 4.4).

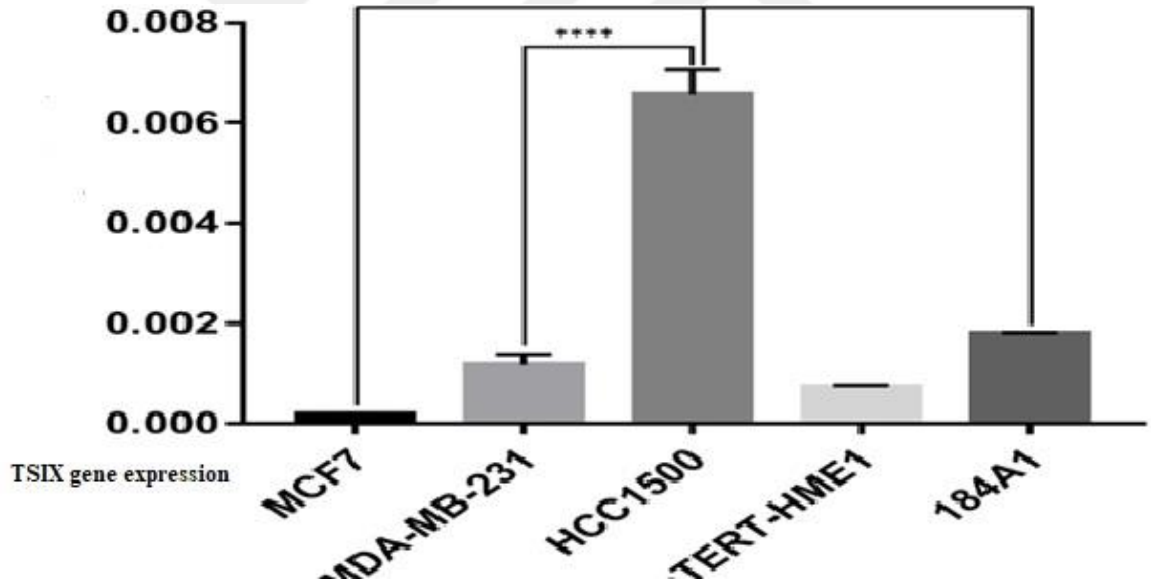


Figure 4.11. TSIX has been shown to be expressed mostly in breast cancer, and the control groupwas found to show the expression of cancer and normal cell line.

Table 4.4. Statistical analysis of TSIX gene in breast

MCF7 vs. HCC1500	-0.006386	-0.007414 to -0.005359	Yes	****	<0.0001
MCF7 vs. hTERT-HME1	-0.0005364	-0.001564 to 0.0004914	No	ns	0.3938
MCF7 vs. 184A1	-0.001616	-0.002644 to -0.0005887	Yes	**	0.0072
MDA-MB-231 vs. HCC1500	-0.005402	-0.006429 to -0.004374	Yes	****	<0.0001
MDA-MB-231 vs. hTERT-HME1	0.0004484	-0.0005794 to 0.001476	No	ns	0.5560
MDA-MB-231 vs. 184A1	-0.0006316	-0.001659 to 0.0003962	No	ns	0.2624

4.7. The expression level inNOV2AS5 of lung

The expression level of NOV2AS5is shown in 2 different cell lines. Gene expression analyses were performed by Real-Time PCR methods (Figure 4.12). NOV2AS5has been shown to have the highest expression level in A549 cells following expression in the A549 cell line, the most among cell lines. NOV2AS5was found to be the lowest in BEAS-2B, control group (Table 4.5).

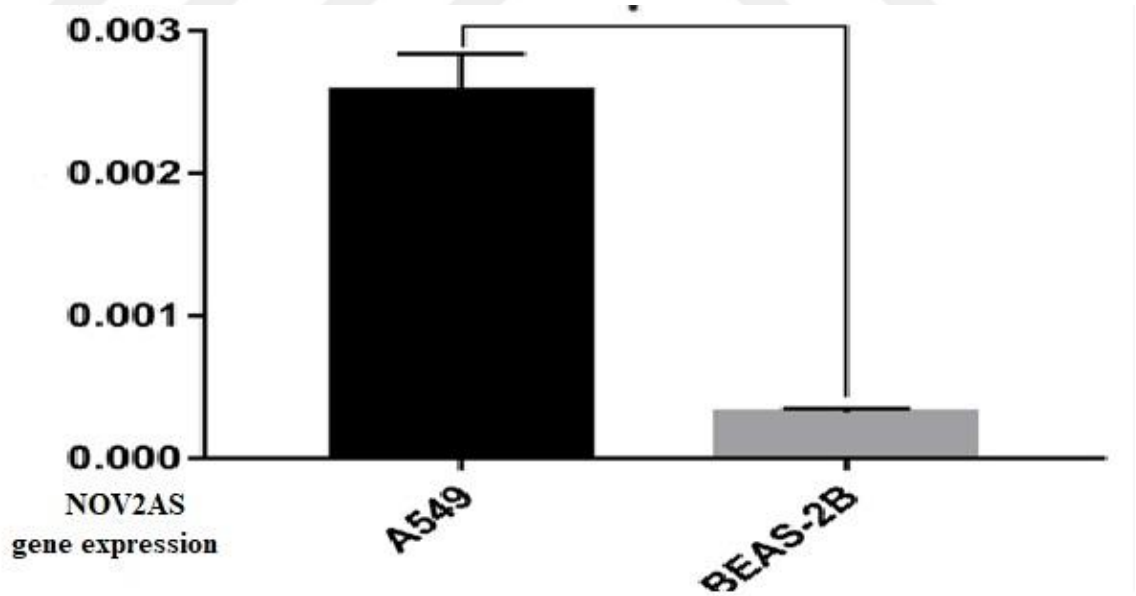


Figure 4.12. NOV2AS5 has been shown to be expressed mostly in lung cancer and the control group was found to show expression of cancer and normal cell line.

Table 4.5. Statistical analysis of NOV2AS5 gene in lung

P value	0.0443
P value summary	*
Significantly different (P < 0.05)?	Yes

4.8. The expression level in NOV2AS5 of breast

The expression level of NOV2AS5 is shown in 5 different cell lines. Gene expression analyses were performed by Real-Time PCR methods (Figure 4.13). NOV2AS5has been shown to have the highest expression level in CRL2329 cells for breast cancerfollowing expression in the MCF7,MDA-MB-231,CRL2329, and CRL4010 cell line, the most among cell lines.NOV2AS5was found to be the lowest in MCF7,MDA-MB-231,CRL2329,CRL4010, control group, and cancer group (Table 4.5).

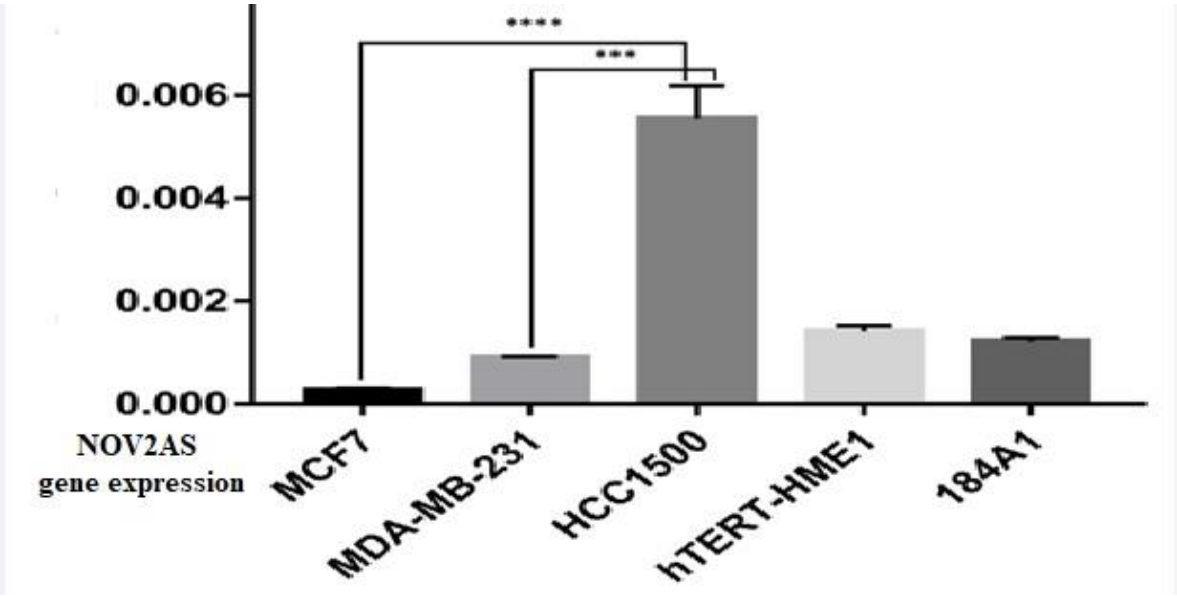


Figure 4.13.NOV2AS5 has been shown to be expressed mostly in breast cancer, and the control group was found to show the expression of cancer and normal cell line.

Table 4.6. Statistical analysis of NOV2AS5 gene in breast

MCF7 vs. HCC1500	-0.005282	-0.006515 to -0.004049	Yes	****	<0.0001
MCF7 vs. hTERT-HME1	-0.001149	-0.002382 to 8.487e-005	No	ns	0.0660
MCF7 vs. 184A1	-0.0009476	-0.002181 to 0.0002858	No	ns	0.1321
MDA-MB-231 vs. HCC1500	-0.004648	-0.005881 to -0.003414	Yes	***	0.0001
MDA-MB-231 vs. hTERT-HME1	-0.0005141	-0.001747 to 0.0007193	No	ns	0.5972
MDA-MB-231 vs. 184A1	-0.0003132	-0.001547 to 0.0009202	No	ns	0.9137

4.9. The expression level of LCMT1AS in lung

The expression level of LCMT1AS is shown in 2 different cell lines. Gene expression analyses were performed by Real-Time PCR methods (Figure 4.14). LCMT1AS has been shown to have the highest expression level in A549 cells following expression in the A549 cell line, the most among cell lines. LCMT1AS was found to be the lowest in BEAS-2B, control group (Table 4.7).

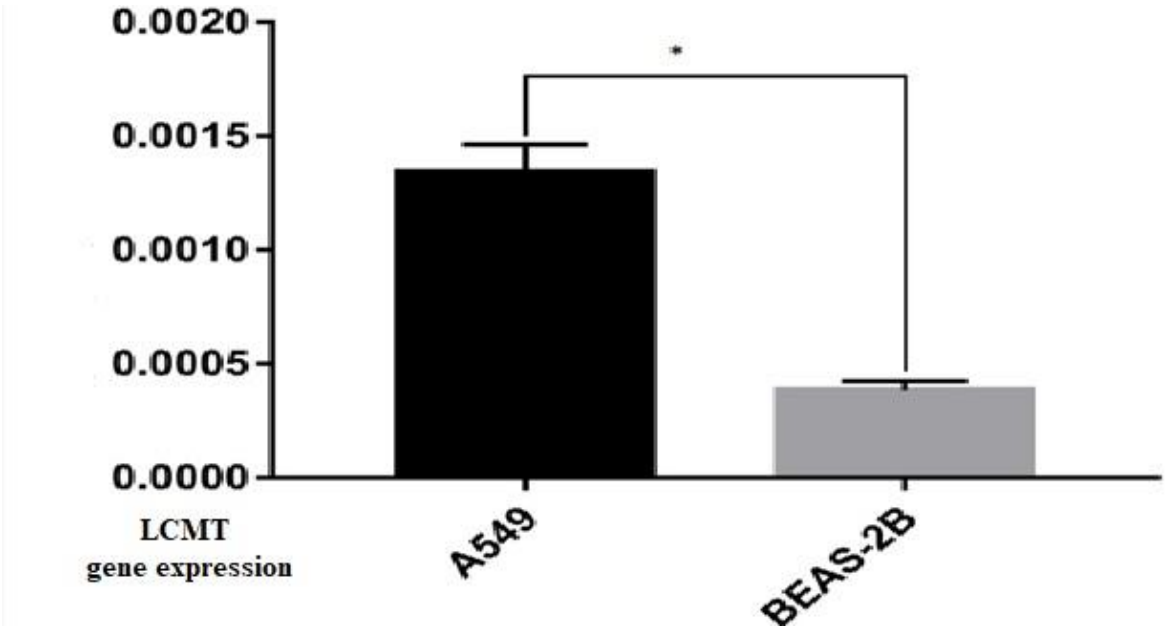


Figure 4.14NOV2AS5 has been shown to be expressed mostly in lung cancer and the control group was found to show expression cancer and normal cell line.

Table 4.7. Statistical analysis of NOV2AS5 gene in lung

P value	0.0443
P value summary	*
Significantly different (P < 0.05)?	Yes

4.10. The expression level of LCMT1AS in breast

The expression level of NOV2AS5 is shown in 5 different cell lines. Gene expression analyses were performed by Real-Time PCR methods (Figure 4.15). NOV2AS5has been shown to have the highest expression level in CRL2329 cells for breast cancer following expression in the MCF7,MDA-MB-231,CRL2329, and CRL4010 cell line are the most among cell lines. NOV2AS5was found to be the lowest in MCF7,MDA-MB-231,CRL2329,CRL4010, control group, and cancer group (Table 4.8).

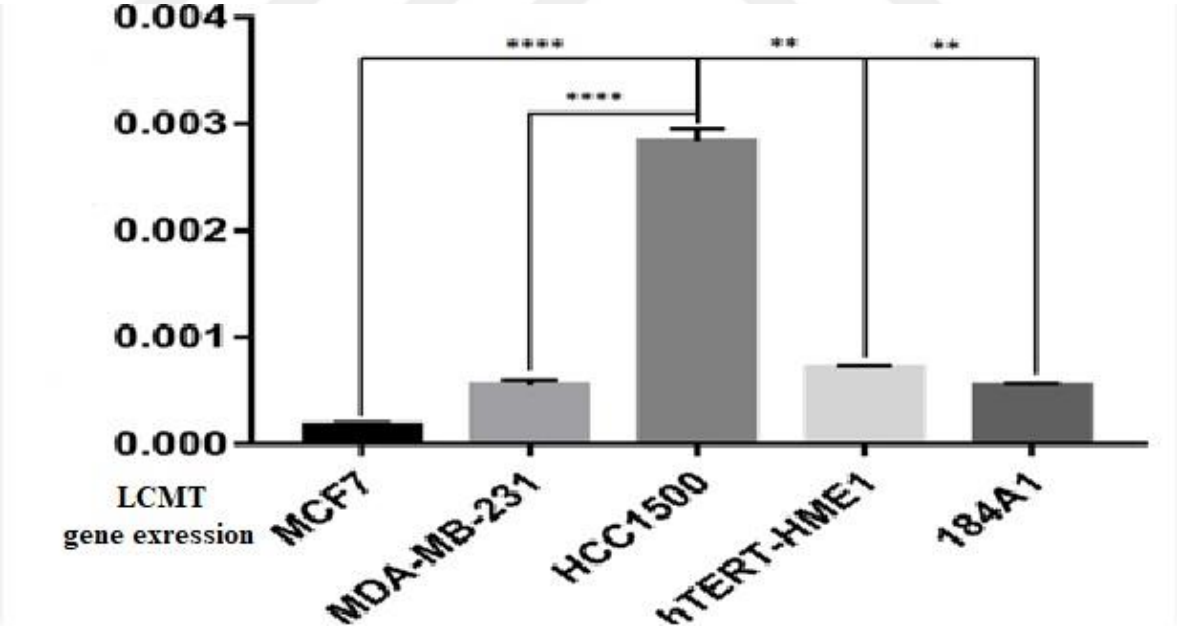


Figure 4.15LCMT1AS5 has been shown to be expressed mostly in breast cancer, and the control group was found to show expression of cancer and normal cell line.

Table 4.8. Statistical analysis of NOV2AS5 gene in breast

MCF7 vs. HCC1500	-0.002664	-0.002903 to -0.002425	Yes	****	<0.0001
MCF7 vs. hTERT-HME1	-0.0005337	-0.0007728 to -0.0002946	Yes	**	0.0014
MCF7 vs. 184A1	-0.0003723	-0.0006114 to -0.0001332	Yes	**	0.0075
MDA-MB-231 vs. HCC1500	-0.002286	-0.002526 to -0.002047	Yes	****	<0.0001
MDA-MB-231 vs. hTERT-HME1	-0.0001565	-0.0003956 to 8.265e-005	No	ns	0.2196
MDA-MB-231 vs. 184A1	4.895e-006	-0.0002342 to 0.000244	No	ns	>0.9999

Real time PCR standadr alaysis

Delta Ct= Ct cancer group - Ct control proup

Delta Ct= ΔCT disease - ΔCT normal

Delta Ct= ΔCT disease (Ct cancer gene -Ct control group) - ΔCT normal (Ct cancer group - Ct control group).

FC= Fold change=$2^{-\Delta\Delta Ct}$

Table 4.9 Delta ΔCT rate for each gene and their P value in breast cancer.

Breast				
Gene name	**Group**	**Normalization**	**Average ΔCT**	**P Value**
TSIX	**Control**	**14.06**	**0.0015**	**0.039***
	Disease	**19.43**	**0.0058**	
HAGLR	**Control**	**25.87**	**0.0028**	**0.004***
	Disease	**26.87**	**0.0010**	
NAV2AS5	**Control**	**12.23**	**0.0029**	**0.002***
	Disease	**20.82**	**0.053**	
LCMT1AS	**Control**	**29.38**	**0.0018**	**0.0001****
	Disease	**25.38**	**0.0023**	

Table 4.10 Delta ΔCT rate for each gene and their P value in lung cancer.

Lung				
Gene name	**Group**	**N**	**Average Δ CT**	**P**
TSIX	**Control**	**22.06**	**0.0043**	**0.0443***
	Disease	**19.55**	**0.0070**	
HAGLR	**Control**	**11.97**	**0.0032**	**0.038***
	Disease	**12.23**	**0.0004**	
NAV2AS5	**Control**	**14.82**	**0.0031**	**0.046***
	Disease	**25.46**	**0.0055**	
LCMT1AS	**Control**	**29.38**	**0.0022**	**0.0005****
	Disease	**25.38**	**0.0034**	

5. DISCUSSION

LncRNAs (long non-coding RNAs), with various important molecular and cellular functions and natural antisense transcripts (NATs), complementary to protein-coding or non-coding RNA sequences are important regulators of eukaryotic gene expression drew a great attention recent years to uncover their importance for diagnostic, prognostic and therapeutic purposes because of their dysfunctions leads to diseases including cancer.This convergence between NAT and lncRNA determination raised confusion and gradually started to be disappeared withthe increasing knowledge. Specific pcGen (protein-coding Gene) regulation by their corresponding ncNATs (non-coding NATs) has been reported. TSIX is a new regulator of collagen expression which stabilizes the collagen mRNA. HOXD-AS1/ HAGLR is a critical regulator for carcinogenesis and metastasis in different types of cancers. LCMT-1 is a negative regulator of Akt proto-oncogene. Not so much information is available related tothe NOV2AS1 gene. This four type of genes regulates cancer cell. We performed a comparison with a control group and cancer groups (70). The tumor suppressor genes(TSIX, HAGLR, LCMT-1, and NOV2AS1)regulate downwards in the cancer cell line(70, 71).

Understanding the molecular mechanisms of cancer cells will be base research for other related investigations and will create opportunities for new cancer therapy especially breast cancer and lung cancer. The strategies regarding investigating these genes related to the cell cycle will be beneficial for understanding the anti-tumoral effect for target organs (72).

TSIX is a tumor suppressor gene. It was identified by the analysis of accumulated transcripts including noncoding histone methyltransferase, which is responsible for trimethylation of the lysine of histone H3 and may play a role in the regulation of mRNA splicing and transcription factor (suppressing tumor development). A number of recent articles correlate loss or gain of function of histone-modifying enzymes, containing histone lysine methyltransferases, with the pathology of lots of types of cancer (73).

Lots of expression profiling studies suggested the potential of gene expression models to distinguish between histologic subtypes effect to expression level from the cancer group and normal group (73).

Gene expression levels of tumor suppressor genes were identified by Real-Time PCR analysis. The seven different types of cell lines of breast cancer and lung cancer were statistically comparable to the control group of their normal cell lines. It has been understood that under the normal condition there was overexpression from cancer cell lines in comparison to the normal group.

The expression level of TSIX, HAGLR, NOV2AS5, LCMT1AS and GAPDH genes in normal breast and cancer cell lines have been demonstrated by RT-PCR analysis. NOV2AS5 has a higher expression than other genes.

In our study, the mRNA expression level of TSIX, HAGLR, NOV2AS5, and LCMT1AS genes was decreased (down-regulated) as shown in Figures 4.10 and 4.12. However, these were statistically significant by Wilcoxon signed-rank test; $p > 0,05$. It was also reported as the downregulated expression of TSIX, HAGLR, NOV2AS5, and LCMT1AS by (74).

In conclusion, the mRNA expression level of TSIX, HAGLR, NOV2AS5, and LCMT1AS genes were significantly differentially downregulated. Understanding the molecular mechanisms of a tumor suppressor of the gene has aided the development of molecular-targeted therapy for breast cancer and lung cancer. In order to understand the molecular mechanisms, we investigated breast cancer, lung cancer, and normal breast. Further analysis is needed.

In this study, mostly mutated regions of TSIX, HAGLR, NOV2AS5, and LCMT1AS genes were analyzed by q PCR roter-gene and sequence analysis. However, expression was observed in these regions. TSIX gene expression was compared from two group disease and a normal group. We analyzed by ImageJ program is comprised of two types of bromodomains in this study such as domain 1; and random region of TSIX, HAGLR, NOV2AS5 and LCMT1AS genes.

We have prepared the groundwork for our next study, tumor suppressor effect or not, we found a result of this, for next study we can check the cell cycle for growth or not,Or we can give siRNA for inhibiting gene expression or give specific miRNA for block gene target.

In the current study, we used four different genes including TSIX, HAGLR, LCMT-1, and NOV2AS1 in breast cancer and lung cancer compared with the normal group. The cancer

samples taken was 5 breast, and 2 from lung cancer cell line. Unless we cultured all taken samples into seven different cell lines include A549, BEAS-2B, 184A1, HCC1500, hTRT-HME1, MCF7, and MDA-MB-231. RNA isolated from used genes for making cDNA, then we performedour experiment by doinga partial-quantitative RT-PCR method for normalisation bands before the beginning of detection of gene expression levels by RT-PCR.

The results showed that the TSIX gene was found mostly expressed in lung cancer and breast cancer. The TSIX gene showed that have the highest expression level in A549 cell lines, while the lowest expression was found in the BEAS-2B cell line respectively. In comparing with breast cancer the highest expression level was found in the HCC1500 cell line, while the lowest expression level was found in MCF7 and hTRT-HME1 cell lines. were measured by Image J software program, the statistical analysis revealed that there are significant differences between the normal group and cancer group cell lines in lung cancer. Another study done by (76), were they used TSIX in breast cancer their outcome disagreed with our results (76).

The results of the HAGLR gene showed that the HAGLR gene was found mostly expressed in lung cancer and breast cancer. The HAGLR gene showed that have the highest expression level in A549 cell lines, while the lowest expression was found in the BEAS-2B cell line respectively. In comparing with breast cancer the highest expression level was found in the HCC1500 cell line, while the lowest expression level was found in MCF7 and MDA-MB-231 cell lines. were measured by Image J software program, the statistical analysis revealed that there are no significant differences between normal group and cancer group cell lines in lung cancer. Our finding was not compatible with another study (77), were they used same cell line, HAGLR expression in another study (78) was upregulated in lung cancer cell which is accordance with our findings, while in study by (77) their result was downregulated in normal cell which is in not accordance with our result (77). The expression level of HAGLR of our study was statistically significant which is strongly aree with (78), but our findings is disagree with (77). Expression level of HAGLR increases with tumour progression and is associated with chemoresistance, tumour metastasis, and diminished patient survival in numerous tumour types including lung cancer (77).

LncRNAs are functionally associated with essential biological processes, including RNA splicing, protein localization, and fatty acid metabolic process (78). Here, we have

demonstrated that repression of TSIX follows silencing of HAGLR. Emerging evidence indicates that FASN play key roles during tumour genesis and development and may serve as a therapeutic target in multiple human cancers (78).

The outcome result of the NOV2AS5 gene showed that the HAGLR gene was found mostly expressed in lung cancer and breast cancer. The NOV2AS5 gene showed that have the highest expression level in A549 cell lines, while the lowest expression was found in the BEAS-2B cell line respectively. In comparing with breast cancer the highest expression level was found in the HCC1500 cell line, while the lowest expression level was found in hTRT-HME1 and 184A1 cell lines. were measured by Image J software program, the statistical analysis revealed that there are no significant differences between normal group and cancer group cell lines in lung cancer.

The result of the LCMT1AS gene showed that the LCMT1AS gene was found mostly expressed in lung cancer and breast cancer. The LCMT1AS gene showed that have the highest expression level in A549 cell lines, while the lowest expression was found in the BEAS-2B cell line respectively. In comparing with breast cancer the highest expression level was found in the HCC1500 cell line, while the lowest expression level was found in MCF7 and MDA-MB-231 cell lines. were measured by Image J software program, the statistical analysis revealed that there are significant differences between the normal group and cancer group cell lines in lung cancer.

Meanwhile, the highest expression level of genes in lung cancer was found in A549 and HCC1500 cell lines, while lowest level expression level of genes in lung cancer was found BEAS-2B, hTRT-HME1, and MCF7 cell lines. However, the highest expression level of genes in breast cancer was found in HCC1500, and A549 cell lines, while lowest level expression level of genes in breast cancer was found MCF7, MDA-MB-231, and BEAS-2Bcell lines.

Functional inactivation of *TSIX* in the context of lncNAT loss of function may represent a key event in facilitating the development of key aspects of a regulator tumor behavior (79). Given the role of TSIX and HAGLR in chromatin modification, the gene expression pathways disrupted by the activation of this antisense may lead to new treatment strategies for different tissues (80).

Epigenetic alterations are the hallmarks of their role in renal tumor development and progression. The last 10 years' reports also suggest that epigenetic alterations play an important role in renal tumorigenesis (76).

The balance between histone acetylation and deacetylation serves as a key epigenetic mechanism for gene expression, DNA repair, developmental processes and tumorigenesis (81). Thus, any reason to make this imbalance can lead to abnormal cell function, even tumorigenesis (81, 82). Volinia*et al,* (82) reported hMOF as an acetyltransferase of H4K16 might be involved in the pathogenesis of renal cell carcinoma, and this epigenetic change might be a new CA9-independent RCC diagnostic marker (783).

A microRNA expression study suggests the involvement in tumor development and tumor progression including metastasis (84). Croce, (85) analysed distant metastases with primary tumors and found a distinct miRNA signature at metastases. Some of the primary tumor samples clustered together with the distant metastasis, suggesting that these primary tumors have a metastasis-specific signature (86).

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