NEAR EAST UNIVERSITY

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THE EFFECT OF NANOBUBBLE OZONE STORED NIOSOMES(NOSN) OF THE WNT/BETA-CATENIN PATHWAY GENES IN MDA-MB-231 BREAST CANCER CELL LINE

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MASTER THESIS IN MOLECULAR MEDICINE

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NEAR EAST UNIVERSITY

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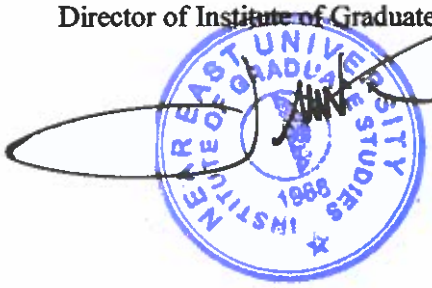

DECLARATION

I hereby declare that all information, documents, analysis, and results in this thesis have been colleted and presented according to the academic rules and ethical guidelines of the Institutes of Graduate Studies, near east University. I also declare that as required by these rules and conduct I have fully cited and referenced information and data that are not original to this study.

Name and Surname: CHRISTELLE KINDUMBA KITOKO

Signature;

Date:

COMPLIANCE AND APPROVAL

DEDICATION

This project is been dedicated to:

My parents, my dad Cleophas KITOKO WANY and my mum EMILIE GOABANA

My uncle JEAN NGANGA

My sisters La JOLIE KITOKO and TATIANA KITOKO

My honey HENCHEAL UCHE

My friends, PAOLLA DIFIMA,PRINSILIA DIBASEMA, GERMAINE NKULU, JOSUE KAYEMBE, ARTHUR BODURIE

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All the glory to our Lord Jesus Christ the master of times and circumstances for the gift of life that allowed me to carry out this scientific project.

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ABSTRACT

THE EFFECT OF NANOBUBBLE OZONE STORED NIOSOMES (NOSN) OF THE *WNT/BETA-CATENIN* PATHWAY GENES IN MDA-MB-231 BREEAST CANCER CELL LINE

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AIM OF THE STUDY: This study aims at analyzing the concentration level of nanobubble ozone expression and complexity, and the destruction of the Wnt/b-catenin signal pathway genes MDA-MB-231 cell-lines.

BACKGROUND Breast cancer is a compilation of clear malignancies that obvious in the mammary glands (Yixiao Feng 2018 Jun). carcinomas constitute the most of breast cancer stint sarcomas such as phyllodes tumors and angiosarcomas are scarcely seen (Yixiao Feng 2018 Jun.). cancer starts when healthy cells in the breast switch and grow out of sway, forming a mass or sheet called a tumor of cells (Asco.org, Cancer.Net editional Board,09/2021).

METHOD AND MATERIALS: Our objective was to treat our MDA-MB-231 breast cancer with nanobubble ozone-stored niosomes (NOSN) (Sonofarma Pharmaceuticals Chemical Industry Trade Ltd Sti, Patent No PCT/TR2022/050177) and to analyze the gene expression profiles of *AXIN*, *GSK3*, *APC*, and *β-catenin* genes. different concentrations of nanobubble ozone were used for 24 hours treatment. Then the molecular analysis including RNA extraction follow up and cDNA synthesis and gene expression analysis were continued.

RESULTS: statistically the genes were grouped into having three varying CT-values respectively *AXIN* 22.42,18.28 and 19.85, *APC* shows CT-values at 20.83,21.85 and 24.62 with *Beta-catenin* having 19.76,22.52 and 17.79 respectively. All three genes had a p values $p<0.05$, and shows they are significant in the study.

CONCLUSION: The goal of this study is to determine how the beta-catenin pathway genes in the MDA-231-MB breast cancer cell line are impacted by nanobubble ozone stored niosomes. These four disting gene sets (*axin,apc,gsk3b* and *catenin* genes) are acquired interchangeably as beta-catenin pathway genes and work in tandem with the

CK1 gene to regulate the Wnt/b-catenin signaling pathway,*AXIN,APC,GSK3*, and CK1 make form the delicate abolition complex. This destruction complex rigorously resolved the amount of beta-catenin because its unwavering focus severely damaged the protein.

KEYWORDS: Breast cancer**,** Wnt/β-catenin signal pathway, nanobubble ozone stored niosomes (NOSN), MDA-MB-231, cell culture

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LIST OF ABBREVIATIONS

μl.: Microliter

mL: Milliliter

μM: Micromolar

WHO: World Health Organization

ERBBS: Erb-B2 Receptor Tyrosine Kinase 2

MDM2: Proto-Oncogene

Bax: BCL2 associated x, apoptosis regulator

P53: Tumor protein

GDP: Guanosine Diphosphate

GTP: Guanosine Triphosphate

VEGF: Vascular Endothelial Growth Factor

FGF: Fibroblast Growth Factor

HGF: Hepatocyte Growth Factor

BER: Base Excision Repair

NER: Nucleotide Excision Repair

DAPK: Death-Associated Protein Kinase

APC: Professional Antigen Presenting Cells

HRT: Hormone Replacement Therapy

PTEN: Phosphatase and Tensin Homolog

PALB2: Partner and Localizer of *BRCA2* (tumor suppressor gene)

TP53: (*TP53* gene provides instructions for making a protein called tumor protein p53)

MBC: Minimum Bactericidal Concentration

STK11: Serine/ Threonine Kinase 11 (tumor suppressor gene)

CDH1: Cadherin-1 or Epithelial Cadherin (E-cadherin)

ER: *Estrogen Receptors*

RAC3: Ras-Related C3 botulinum toxin substrate 3

GHRHR: Growth Hormone (GH)-Releasing Hormone (GHRH) Receptor (GHRHR)

RAS: Rat Sarcoma Virus

MYC: Master Regulator of Cell Cycle Entry and Proliferative Metabolism

PTEN: Phosphatase and Tensin Homolog

ADAM: A Disintegrin and Metalloproteinase

WNT: Wingless-Related Integration Site

TCF/LEF: T-Cell Factor/Lymphoid Enhancer Factor

FAP: *Familial Adenomatous Polyposis*

CKI: Cyclin-Dependent Kinase Inhibitor

GSK3: Glycogen Synthase Kinase *(GSK)3*

HCC: Hepatocellular Carcinoma

SOX7: SOX7 is a Transcription Factor and acts as a tumor suppressor

SMAD7: Mothers Against Decapentaplegic Homolog 7

AKT: Protein Kinase B, also known as Akt

HIV: Human Immunodeficiency Virus

SARS: Severe Acute Respiratory Syndrome

ROS: Reactive Oxygen Species

OIS: Oncogene-Induced Senescence

PDH: Pyruvate Dehydrogenase

BRCA-1 gene: Breast Cancer type 1 gene

BRCA-2 gene: Breast Cancer type 2 gene

caspase-3: Protein product of *CASP3* gene., member of cysteine-aspartic acid protease (caspase) family.

DMSO: Dethyl Sulfoxide

E-cadherin: Cell adhesion molecule E

EGFR gene: Epidermal Growth Factor Receptor

EDTA: Ethylenediaminetetraacetic Acid

FBS: Fetal Bovine Serum

GSH: Reduced Glutathione

HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HER-2/Neu: Human Epidermal Growth Factor Receptor 2/ proto-oncogene Neu

HIPK2: Homeodomain Interacting Protein Kinase 2

MCF-7: Breast cancer cell line isolated by Michigan Cancer Foundation

MDA-MB-231: Breast cancer cell line isolated by M. D. Anderson

Mg: Magnesium

MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

p53: *TP53*, Type of tumor suppressor gene

P450: Cytochrome *P450* gene

PC12: Cell line from a pheochromocytoma of the rat adrenal medulla

PR: Progesterone Receptor

RCF: Revolutions per minute in a centrifuge

ROS: Reactive Oxygen Species

SH-SY5Y: Neuroblastoma cell line

Th 1, Th 2: Helper cells

CHAPTER 1: INTRODUCTION

1.1 INTRODUCTION

1.1.1 BREAST CANCER

Breast cancer is a compilation of clear malignancies that obvious in the mammary glands (Feng, 2018a). carcinomas constitute the most of breast cancer stint sarcomas such as phyllodes tumors and angiosarcomas are scarcely seen (Feng, 2018a). cancer begins when healthy cells in the breast switch and grow out of sway, forming a mass or sheet called a tumor of cells (Cooper M. Geoffrey, 2000) A tumor may be benign or malignant, a malignant tumor is one that spreads to other bodily parts and grows rapidly. A benign tumor is one that is still growing and has not yet spread (Cooper M. Geoffrey, 2000). Giving to data from 2020, the asir was highest in very low human development index (HDI) countries (75.8 per 10.000), while it was at the bottom of the list (Lukasiewicz, 2021a). In addition to being the most common, breast cancer is the leading cause of cancer death in women worldwide. The age-adjusted incidence rate of breast cancer was 13.6/100.000 worldwide (95% UI, 675, 493-694, 633) (Lukasiewicz, 2021a). Notwithstanding the high occurrence rates in industrialized countries, 63% of all deaths in 2020 will occur in Asia and Africa. The majority of women with breast cancer in cost effective nations will survive; however, this is not the case for women in the majority of impoverished and many middle-income nations (Lukasiewicz, 2021b). Numerous significant unresolved clinical and scientific issues persist despite notable advancements in the analysis and treatment of breast cancer (Polyak, 2007b).

Worlwide, parent inherited gene alterations are linked to 5-10% of cases of breast cancer (Feng, 2018a). Specially, women with breast cancer gene (BRCA1) mutation have a 55-65% lifespan probability of spreading ductal carcinoma (Feng, 2018a). Inherited mutatios in breast cancer genes1 (BRCA1) or breast cancer gene2 (BRCA2) are the most common cause of hereditary breast cancer. The life probability is 45% for women who have the breast cancer gene 2 (BRCA2) mutation According to (Feng, 2018a), women with the BRCA1 or BRCA2 gene mutations had a 70% probability of developing breast cancer by time they are 80. The likelihood of breast cancer increases as more family members are affected by the influence of mutation, according to (Feng,

2018a). Although anyone can have these mutations; jews of Ashkenazi (Eastern European) radix are more likely to have the breast cancer gene (BRCA) alterations in the us than persons of others racial and ethnic groups are (Feng, 2018b). Additionally, women who carry one of these mutations are more likely to get breast cancer early in life and to develop the disease in both breasts (Feng, 2018b).

The impact of the breast cancer gene1 (BRCA1) and breast cancer gene 2 (BRCA2) mutations widens pqst just breast cancer as having mutations in either of these genes aliked with a heighten ovarian cancer risk as well (Feng, 2018b). Inversely, breast cancer gene (BRCA) mutations are being less regularly in breast cancers happening in men breast cancer gene 2 (BRCA2) mutations are kindred with a life breast cancer risk of only roughly 6.8% (Feng, 2018b).

Wellings and Jensen (1973) as well Wellings et al., (1975). Challenged the accepted theory that unique microanatomical features of the normal breast would give rise to notable histological type of breast cancer. The terminal duct-lobular unit is the primary source of invasive breast cancers.The terms ductal,lobular carcinoma should be stressed since they allude to the mammary ductal system as the histogenesis or location of origin these are entities, not individuals that are identified based on cytological traits, immunohistochemical profiles, and disconnected architectural patterns. The authors cite a current review by Yerushalmi et al., 2009 for a detailed discussion of the histology of certain forms of breast cancer.

the landmark learning by, Preou et al., (2000). cDNA microarray analysis was performed on 38 invasive breast cancers, of which two were invasive lobular carcinomas and 36 were invasive ductal carcinomas. We used one fibroadenoma, one ductal carcinoma and three norm al breast samples to determine an intrinsic gene list, multiple biological replicates from the same patient are used. In studying the transcriptome of breast cancer, oestrogen receptor (ER)-positive and ER-negative breast cancers were distinguished in the cluster dendrogram of (Weigelt et al., 2010; Van't Veer et al., 2002.; Correa Geyer and Reis-Filho 2009.; and Gruvberger et al 2001). Using this intrinsic gene list, the same team was able to show that the ER-positive luminal group can be divided into at least two subclasses, luminal A and luminal B, and that the different molecular subtypes were associated with different clinical outcomes in a subsequent study with a larger cohort of patients. These

molecular subgroups of breast cancer were created and formed in subsequent microarray datasets (Sorlie et al., 2003.; Christos Sotiriou et al., 2003., Zhiyuan Hu et al., 2006) and some enlarge by multiple groups.

1.1.2. RISKS AND FACTORS OF BREAST CANCER

Due to proven hereditary predisposition, about 1% of women have a very large possibility of spreading breast cancer. The occurrence od cancer in the early stages is frequent (Lukasiewicz, 2021b). Bilateral prophylactic mastectomy is a highly effective treatment option for these women (Alaofi, 2018). It is even less acceptable for women who have a strong family history but an inadequately determined mutation status, because they cannot know whether their lifespan chance an evolving breast cancer is subordinate to that of the general population (10% to 85%) or higher than that of a mutation carrier (about 85%) (Metcalfe and Narod, 2009). The cure rate for pre-invensive or prematurely invensive malignancies must be >90% for lifelong observation to be a suitable option to mastectomy. While the particular determining factor of breast cancer is undisclosed, one of the well-established predictors of risk is family history, which may indicate hereditary elements (Polyak, 2007b). When they attacked relatives who were under 50 years old, the risk may even have increase (Lukasiewicz, 2021b).

Due to the heightened hormonal stimulation, fetus sex is one of the main components connected to a higher chance of breast cancer (Lukasiewicz, 2021c). Premenopausal and postmenopausal women take a bigger chance of breast cancer due to changes in the physiological stages of endogenous sex hormones; these observations were as well reinforced by the endogenic hormones and breast cancer combining group (Lukasiewicz, 2021c). Males account for less than 1% of all breast cancer cases. Although it is a rare disease breast cancer in men tends to be diagnosed earlier than in women (Lukasiewicz, 2021b).Numerous genes have associated with breast cancer tumor growth and dissemination are significantly influenced by oncogene and anti-oncogene alterations as well as aberrant amplification (Sun et al., 2017).

Breast cancer genes 1 and 2 (BRCA1 and BRCA2) genes are two known anti-oncogenes for breast cancer chance. The genes, positioned on chromosome 13q12 and

17q21 correspondingly, mutually produce proteins that inhibit tumour development. According to (Sun et al., 2017), BRCA1 deletion leads to abnormal centrosome duplication, genomic instability, cell cycle checkpoint disruption and eventually death, with pocket proteins for instance p107,p130 and the retinoblastoma protein suppressing BRCA1 epression in E2F-reliant way. Breast cancer chromosome 1 (BRCA1) has been shown to set up a circle among the booster, Introns and exterminator sections that interacts with its own promoter to drive expression of this gene (Sun et al., 2017). By interrelating through RAD51 and DMC1, breast cancer-associated gene 2 (BRCA2 proteins) controls recombinant repair in DNAdouble-strand breaks (Sun et al., 2017).

Despite having a luminal character, high-grade invasive ductal carcinomas are more common in breast cancer associated gene 2 (BRCA2) associated breast cancer tumors (Sun et al., 2017). A person's risk of evolving breast cancer can be significantly amplified if they are carrier of a harmful mutation in the breast cancer gene 1 (BRCA1) or breast cancer gene 2 (BRCA2) , which have been linked to the disease. The breast cancer gene 1 (BRCA1) and the breast cancer gene 2 (BRCA2) transformations are congenital in a somatic chromosome governing manner, although thesecond allele is normal. 5 to10% of all breast cancers are caused by cancer of breast-linked genes 1 and 2 (BRCA1 and BRCA2), according to 2017 research by Yi-Sheng Sun et al.

According to Chen's meta-analysis, 57% and 49%, respectively, of women older than 70 who have breast cancer associated ga 1(BRCAR1) or cancer of breast associated chromosome 2 (BRCA2) mutations are breast cancer carriers (Sun et al., 2017).

The danger of cancer of breast can be augmented through modern lifespan choices such extreme alcohol usage and nutritional fat ingesting. Alcohol consumption could activate estrone receptor pathways and increase the levels of hormones linked to estrogen in the blood (Sun et al., 2017). A meta-analysis founded on 53 epidemiologic researchs found that consuming 35 to 44 grammes of alcohol per day could rise the chance of breast cancer by 32%. The same meta-analysis also found that for each extra 10 grammes of alcohol spent per day, the danger rises by 7,1%.

The current western diet is associated with excessive consumption of fat, especially saturated fat, according to Yi-Sheng Sun et al (2017). According to Yi-Sheng Sun et

al (2017), both alcohol consumption and smoking growth a woman's risk of starting breast cancer (RR=1.54). Currently, there is growing evidence that smoking, especially if started at a young age, increases the probability of developing breast cancer (Sun et al., 2017).

1.1.3. CELL CULTURE OF BREAST CANCER

Breast cancer endures to be the subsequent greatest mutual basis of cancer in females. Long-term survival will increase with knowledge of breast cancer recurrence's nature (Lukasiewicz 2021c). It should be mentioned that numerous alternations in the genetic and molecular pathway implicated in breast cancer have been seen throught time, lowering the death rate. (Feng, 2018c). However, even 20 years after the first effective therapy, the majority of patients still experience recurrent tumors at local or distant sites that are frequently incurable (Moletta, 2019). Reseachers from the 3D breast cancer(modelling breast cancer in 3D cell culture systems and animals) project, which was financed by the European union, tackled this issue by learning the mechanisms behind tumor theory, cell escape, and cell survival.(Leonard and Godin, 2016).

They are dedicated to developing novel models that accurately represent real disease as well as enhancing clinical therapy choices (Masic, 2008). By concentrating efforts on animal model systems that have made it possible to identify and separate primary mouse mammary epithelial cells, the researchers were able to generate 3D organotypic cell cultures and examine the organization at specific phases of tumor formation as a result of them. Position and cell fate at the single cell level in relation to the overall in vivo phenotype (Mills Shaw and Brugge, 2004).

The comparison with normal mammary stem to identify the origin of the cells initiating the tumor, while opening avenues for treating the tumor (Zhang and Li, 2020). Specifically, an emphasis was placed on the mechanisms keeping dormant residual tumor cells alive and on the molecular properties that facilitate tumor recurrence.

To prevent breast cancer relapse, researchers have explored these cells' dependence on oncogenic factors. Additionally, a new method for researching tumor-initiating cells and understanding the underlying mechanisms of relapse has been made possible by the 3D breast cancer culture system. (Bushenell, 2021).According, to the study's

findings will identify new targets that can be used in the clinical context to increase breast cancer patients' chances of survival (Jiao, 2014). It should be noted that a cell culture is an experimental approach that has been widely used since the beginning of cancer research.

However, this method has contributed a significant amount of knowledge about the biology of cancer cells and is still frequently a necessary step. However, there are additional types of cultures, such as diploid primary, secondary, or continuous cell lines. (Mirabelli, 2019).

Freshly separates tissue cells that are proliferating under the correct conditions are used to establish primary cultures.(Alberts B, J. A. L. J. et al., 2002).

Despite the fact that cell cultureis a crucial tool for biotechnologies. These replicas aren't like the originals bacteria, yeasts, or cells derived from animals may be used as these in vitro models. Reconstructing the original circumstances of the cellenvironment is necessary.(Verma, A., Verma, M., and Singh, A, 2020b)). In order to achieve this, we must regulate the plant cells PH, temperature, pressure, humidity, and mineral and nutritional content. In vitro cultivation of plant cells is another option, explants of leaves, stems, bubs, fruit ovaries, anthers, roots are used tocreate cultures (Huayi, Li, 2020).

1.2. CHARACTERISTICS OF MDA-MB 231 CELL LINES

Over 90% of the MDA-MB-231 breast cancer cell line's population expresses CD44+/CD24low, which is triple-negative cell line with a mesenchymal phenotype (Thomas Hero, 2019). regarding the conclusions reacged by Gupta et al.

Anti-hormonal and targeted therapy are unpromising for triple negative breast cancer since the cells do not express oestrogen or progesterone receptors or have an excess of HER2.(Van, Barele, 2021).

One of the well document triple negtive breast cancer cell line (TNBC) MDA-MB-231 was created from a pleural effusion in 1970s (Kathryn, J Chavez et al.,2010). in addition to having the aggressive nature of all triple negative breast cancer (TNBC) cells, MDA-MB-231 cells have mutant p53, which more difficult (Huang, 2020).

Triple negative breast cancer (TNBC) MDA-MB-231 cells exhibit typical epithelial mesenchymal transition (EMT) linked to development of cancer, there aren't any thorough desceiptions of the structure and biological traits of MDA-MB-231 spheroids, which calls for more study (Huang 2020).

1.2.1. MDA-MB-231 CELL LINE

TNBC is a breast cancer subtype that is aggressive and has few available therapeutic options (Li Yin et al 2020). Due to the drug-resistant nature of their metastatic burden, TNBC patients have a 77% 5-year survival rate compared to those with less aggressive breast cancers (Przanowski, 2020).

Ahead of this time, mouse models have been developed whose goal is the discovery of fresh treatment approaches. reducing TNBC tumour development and metastatic spread (H Charles Manning., Jason R., Buck., and Rebecca S. Cook., 2016). However, MDA-MB-231 breast cancer cells were put in the fourth breast fat pad and described as a practical guide for an orthopaedic example of TNBC, closely mimicking the behaviour of cancer cells in humans (S.Cheng,2020). From udy, the evaluation of lung metastasis using in vivo and ex vivo imaging, as well as molecular detection, is described. Tumors are measured using callipers (Renske J.E van. Den Bijgaart, 2016). In particular, it is appropriate for evaluating the communication amid the main tumour and distal metastatic locations. This model by itself offers a great platform for examining therapy efficacy (Pouliot and Burrows, 2013).

1.2.2. MOLECULAR PROFILE OF MDA-MB 231 CELL LINES

TNBC is regarded as an aggressive subtype of breast cancer with few available therapeutic options (L.Ying, 2020). The 5-year survival rate of TNBC patients with less aggressive breast cancers is expected to be 77%, which is accounted for by their drug-resistance phenotype and metastatic burden (Qitong Wu., Sumit Siddharth., and Dipali Sharma 2021).

To put an end to this, mice models have been developed in an effort to find novel therapeutic approaches that restrict the growth and metastatic spread of TNBC tumours (Xiyun Deng., and Ying Li., Zhijun Zhan., Xuemin Yin., Shujun Fu., 2021).

Nevertheless, studies have aided in describing the aforementioned procedure and providing instructions for the orthopaedic model of TNBC, which involves implanting MDA-MB 231 breast cancer cells deferred in a basement membrane matrix into the fourth pad of mammary fat and closely simulating the behaviour of cancer cells in humans (Cheng, 2020). It is debatable if using callipers to measure tumours or use in vivo and ex vivo imaging to assess lung metastasis and molecular detection (Renske., J.E. Van., Den., Bijgaart, 2016). However, this theme offers a superb framework for the pertinent and specific research necessary to examine the relationship between the main tumour and the distal metastatic sites (Gupta and Massagué, 2006).

Nonetheless, cell cycle balance is governed by two types of genes involved in carcinogenesis: cell division is stimulated by pro-oncogenes and cell division is inhibited by anti-oncogenes (Zhang, 2010). Note that a mutation in these genes, activating a proto-oncogene or inhibiting an anti-oncogene, can also cause the cell to multiply in anarchy and infinite manner, overriding the controls of the cell (Kufe 2003a).

Furthermore, the mechanism by which a parent cell divides into two identical daughter cells is called a cellular cell, its duration varies depending on the cell type, and it is generally between 10 and 24 hours for eukaryotic cells (Khand academy, 2023). Indeed, in several breast cancer cell lines and interaction has however been demonstrated, citing BT-20, ZR-75-1, MT3, MDA-MB-231, or even the MDA-MB 435 line used in mistaken for several years as a breast cancer line but being the M14 melanoma line (Prasad and Gopalan, 2015).

However, one of the main tools in preclinical studies in oncology is cell lines (Pharmalegacy.com, preclinical studies in oncology and immune-oncology). Over the past 40 years, vast amounts of data relating to breast cancer have been obtained, the first lines were established and very few lines developed in the 1960s and 1970s (Liu. Edison.T, 2000).

In addition, there are around 100 such lines for breast cancer, and three of them are widely used (Dai.,Cheng., Bai., and Lia., Li., 2017). These three lines (MCF-7, MDA-MB 231, and T47D) are cited in a statistic of about 80% of publications that mention breast cancer cell lines (Dalla and Lowe, 2020). These lines are mainly differentiated

by their estrogen and progesterone receptor status (EGR/PGR/HER2) (Sperduto, 2020).

By establishing the use of these cell lines several advantages arise such as complete control of environmental conditions and a standardized nutrient medium; significant reproducibility of experiments; a possibility of cryo-preserving the strains in the very long term; (Segertz and Vallier, 2017) A lower cost than the use of vivo models and fewer ethical issues compared to in vivo models (Polli, 2008). While on the disappearance of cells from the surface of the gel, an observation was made. The MDA-MB 231 and MCF-7 lines are lines derived from mammary carcinoma and the LS180 AND HT29 are colon cancer cells (SSM. Petersen, 2021). MDA-MB231 disappears more quickly from the gel surface than MCF-7 suggesting a greater invasive potential (Sun, 2016).

1.2.3. MDA-MB 231 CELL LINE ANGIOGENESIS AND LYMPHOMAGENESIS

Angiogenesis in cancer is essential for tumour growth and spread and is initiated by chemical signals from rapidly developing tumour cells (Folkaman, 1971). Muthukkaruppan and colleagues (1982) evaluated the behaviour of cancer cells put into the same organs from various organ locations in a previous investigation. The iris was one area with blood flow, and the anterior chamber was another with no blood flow (Naoyo, Nishida; 2006). Without blood circulation the cancerous cells would have reached a diameter of 2 mm3, then if there were stops, but exceeding 2mm3 when they were placed in an area where angiogenesis was possible (Naoyo, Nishida, 2006). Tumors that lack circulatory supply may develop necrosis or even apoptosis (Naoyo. Nishida, 2006).

Neovascularization, which includes tumour angiogenesis, is essentially a four-step process, and as a result, angiogenesis is a noteworthy role in the development of cancer (Naoyo, Nishida, 2006). The tissues' localised basement membrane is first damaged. The angiogenic process begins with rapid destruction and hypoxia, followed by endothelial cells that have been triggered by angiogenesis factors migrating, proliferating, and stabilising. Vascular endothelial cells typically only divide every 1000 days (J Denekamp, 1993).

Angiogenesis is triggered when the tumour tissues want nutrition and oxygen. Angiogenesis is controlled by activator and inhibitor molecules (Nayo Nishida 2006). In reality, the angiogenesis of the tumour cannot occur without an increase in the activity of angiogenic factors (Folkaman, 1991). Inhibitors of vessel development or negative regulators should also be downregulated (Naoyo, Nishida, 2006).

1.3. GOOD AND BAD ANGIOGENESIS (Abnormal and normal)

When new blood vessels are visible, angiogenesis can be a natural, healthy biological function (Tahergorabi and Khazaei, 2012). This occurs in the context of child growth, when the endometrium in women empitied each month during menstruation and new blood vessels are needed for healing (Critchley 2020). Researchers looking for ways to stimulate angiogenesis in tissues damage for example, after a heart attack may pursue these ideas further (Lynne Eldridge, MD, 2019). The body's various processes depend on a delicate equilibrium. In the case of cancer, angiogenesis—the growth of new blood vessels—allows tumours to form. Any sort of blood vessel is referred to as angiogenesis, which shares the same definition as the word "neovascularization." (Artery, vein, capillary, lymphatic vessel). (Osterby and Nyberg, 1987).

1.3.1 ANGIOGENESIS VASCULOGENESIS

There are numerous words that describe the development of blood arteries, each with distinct meanings. The standard for angiogenesis is the utilisation of existing blood vessels. On the other hand, vasculogenesis describes the Novo (original) development of blood arteries in the embryo. Angioblasts, which are immature cells that develop into endothelial cells, are the source of these new blood arteries. However, other studies imply that vasculogenesis might contribute to the development of some malignancies (Dudley, 2012).

1.3.2. THE ROLE OF ANGIOGENESIS IN CANCER GROWTH

Angiogenesis is of importance to the cancer community since the development of new blood vessels is compulsory for the growth and metastasis of malignancies (Lugano, 2020b). Angiogenesis must take place to allow cancers to grow to a size of approximately one millimeter (0.2mm) (Ribatti, 2008). Cancer accomplishes this

through secreting chemicals that promote angiogenesis and, thus, the growth of the disease (Rajabi and Mousa, 2017).

1.3.2.1. ROLE IN METASTASIS (SPREAD)

Its role is to be a crucial process in the spread of cancers and their invasion of neighboring tissues, the importance of angiogenesis is for the production of metastases (Bielenberg, 2015). Noting that these cells must conduct new blood vessels to carry their progress in new place, cancer cells must travel and settle somewhere other than their original location (Shenoy, 2016).

1.3.2.2. PROCESS OF ANGIOGENESIS

Before that, it is figured that blood vessels have to enlarge and turn into more porous, begin formation, grow (proliferate) in order for angiogenesis to begin (Adair et al., 2010), Migration and differentiation tube creation (maturation) Additionally, pericytes, which are important for maintaining new blood vessels, are recruited by malignancy (Bergers and Song, 2005). The tumour microenvironment, or the healthy tissue that surrounds a tumour, plays a critical part in the delicate regulation of the entire process by proteins that can tip the scales in either direction (Watnick, 2012).

1.3.2.3. REGULATION OF ANGIOGENESIS

There are numerous angiogenesis-activating and -inhibiting proteins, to use the vascular endothelial growth factor (VEGF) example from before. The development of angiogenesis in cancer is thought to require more than only increased activating factor activity, despite the importance of this (Naoyo. Nishida, 2006). Additionally, elements that prevent blood vessel formation ought to be less active than they otherwise would be (Merks and Glazier, 2006).

1.4. MDA-MB 231MESENCHYMAL INTERACTIONS

Mesenchymal stromal cells and cancer are they hope or fear? The development of cancer includes not only the growth of the primary tumor but also, in some cases, the dissemination of cancer cells in the body and the appearance of metastases in various target organs (Galland and Stamenkovic, 2020). At the end of the 21st century, Stephen Paget issued an opinion on a hypothesis according to which this model imperatively generates a close relationship between cancerous cells and components constituting

the tumor microenvironment (Langley and Fidler, 2011). And the latter contains various cell types: endothelial cells, cells of the immune system (B lymphocytes, T lymphocytes, neutrophils, macrophages, and dendritic cells), adipose cells, but also mesenchymal stromal cells (MSC) (Li,Peishan, 2019).

This combination slows down or accelerates tumor growth and affects the proliferation/cell death balance via the secretion of growth factors, cytokines and chemokines, and proteases. In the same way, these factors will modulate the vascularization of the tumor (angiogenesis), allowing it to escape the immune system and promoting the ability of tumor cells through the basement membrane to eventually metastasize (Lugano, 2020a).

demonstration of recent studies shows that, among all the cells present in the tumor a microenvironment, mesenchymal stromal cells could play a main role in tumor growth (Nwabo,Kamdje, 2017). We should also note that the description of the first mesenchymal stromal cells in 1996 by freindestein from bone marrow cultures, as cells adhering to plastic, capable of cloning and proliferating rapidly. (Chen, 2007).

1.5. PROLIFERATION OF BREAST CANCER

Concerning breast cancer, the proliferation of cancerous cells is under the influence of different substances recognized by specific receptors: hormones and EGF (epidermal growth factor, promoting the growth of epithelial cells) (Masuda 2012). Note that the BRCA antigen (breast cancer antigen) 10% to 5% of breast cancers are linked to a genetic abnormality (mutation) of a gene called "BRCA", according to statistics (Petrucelli, 2022).

The transmission of the said anomaly by the hereditary way and highlighting by genetic screening. 85% of breast cancers are caused by malignant cells in the milk ducts. Globular carcinoma: the presence of cancerous cells in the lobules, represents 10%-15% of breast cancers (Feng, 2018d). Thousands and billions of cells grouped to form tissues and organs are the compositions of the human body (Khan, 2021).

Thus, in the nucleus of each cell, there is the presence of genes that indicate to the latter the moment of its development, of its work, of dividing and dying. Normally we say healthy because the cells follow all these guidelines, a gene can mutate when

modifying or damaging our DNA. Remember that gene mutations do not function properly because the directives in their DNA are confused (Wiley.Liss, 2002)

While the rest of the cells could cause it to divide and grow in a disorderly way, which can lead to cancer (Cooper G.M, 2000).

Generally, in adults, cells grow and divide to only produce more cells when the body needs them, this is the replacement of ageing or damaged cells (Xing and Chen, 2008). Thus, there is a certain difference between cancer cells because they have genetic mutations that change the normal cell into a cancer cell (Eldridge, MD, 2023). Let's remember that genetic mutations can be hereditary, flourish over time as we get older and the genes are exposed to something that damages our genes, such as cigarette smoke, alcohol, or the ultraviolet rays of the sun (Sun et al., 2017).

The malignant cells behave differently in the normal cell. Its division and expansion begin in an erratic manner rather than when they ought to. Additionally, it does not mature as much as a normal cell, leaving it immature. Cancers come in a variety of forms, and they all arise when cells develop abnormally and uncontrollably (Cooper G.M, 2000).

1.6. SPHEROID FORMATION

Spheroids are cellular aggregates that can resemble tissues and microtumors in three dimensions (3D) (Honglin, Shen, 2021). Significant progress has been achieved recently in creating tumour cell aggregates that can be used as models for in vivo tissue settings (Fontana,F, 2021).

These aggregates will form a recognizable spheroid when planted into a microplate well with a circular bottom and very low blinding (Leung, 2015). Spheroids are also regarded to behave more like tumors than conventional two-dimensional (2D) culture because, like tumors, they contain both deeply embedded cells and cells that have rxposed surface (Nath and Devi, 2016c).

Having cell that are both proliferating and do not proliferate, a hypoxic center,and a layer of cells on the are highly oxygenated (Hubbi and Semenza, 2015). Remember that these 3D spheroid models are successfully used In scrrening settings to improve medication safety assessments and identify new cancer treatmentagents.

Understanding the development of tumor cells is one of the most crucial goals in cancer research (N, Jackson, Bromma, 2022).

Solid cultures, which are muche more precise, allow for the visualization of the physiological changes that separate healthy cells from malignant cells (Nath and Devi, 2016c). In order to better understand the tumor microenvironment, researchers can see how tumor cells interact with one another, take in nutrients, and polymerize using multicellular tumor spheroid models (Wu and Swartz, 2014). Naturally, when evaluating cellular responses to therapeutic interventions, spheroids are ideal for preclinical drug testing and validation (Pinto, 2020).

More specifically, they can show whether a medicine can penetrate the tumour and whether it has an inhibitory effect on metastasis (Fontebasso and Dubinett 2015). Spheroids are used extensively in stem cell research, particularly when cultivating neural stem cells and embryonic stem cells (ESCs) (NSCs) (Cesarz and Tamama, 2016).

The promise for regenerative medicine and cell therapies was first suggested by researchers who cultivated mesenchymal stem cells (MSCs) in spheroids and showed their regenerative and anti-inflammatory effects (Han, 2019b). When it comes to 3D cell culture techniques, spheroids have several benefits, such as their applicability to tumour biology, cheaper cost, less labour-intensive than animal models, reproducibility, and simplicity of integration into high-throughput screening and advanced imaging techniques (Habanjar, 2021).

1.7. WTN/B-CATENIN PATHWAYS

Wtn/b-catenin is a protein whose gene is CTNNB1.it plays role in cell adhesion, cell signaling, and gene transcription (MedlinePlus, 2018). In addition, it comprises a central part made of repeated amino acid units, a C-terminal part constituting an alpha helix, and an N-terminal part (Legardinier, 2009). It should be noted that the latter acts as a transcriptional coactivator in the Wnt pathway, it uses this pathway, regulates angiogenesis in the central nervous system, and participates in the formation of the blood-brain barrier (Hübner, 2018).

It is also, with cadherin with which it binds, and alpha-catenin, a constituent of adherens junctions and therefore plays an important role in intercellular adhesion,

particularly at the neuronal level (Mège and Ishiyama, 2017). It intervenes in the remodeling of synapses and thus plays a role in memory. At rest, B-catenin is phosphorylated by GSK3B, which marks it for degradation for the proteasome (Mège and Ishiyama, 2017). In medicine, certain mutations of CTNNB1 are associated with an intellectual deficit, one of these mutations induces an autosomal dominant syndrome associated with hypotonia during childhood, spasticity of the lower limbs, disorders of higher functions, and abnormalities of the facies (Ho.KL and chung, 2022).

Mutations are found in a third of adrenal adenomas, whether malignant or benign, one of these mutations increases the activity of beta-catenin, with significant activation of the Wnt pathway, responsible for elevated aldosterone secretion in adenoma, the Wnt-beta catenin system inhibits the angiogenesis of certain brain tumors such as gliomas (Tissier, 2005).

The Wnt is a family of glycoproteins involved in embryogenesis and cancer. The wingless gene was first identified as a gene involved in morphogenesis in the fruit fly Drosophila melanogaster (Bejsovec, 2018).

All animals' embryogenesis and the homeostasis of adult tissues depend on the Wnt family of glycoproteins, which include roughly 350 amino acids worth of cysteines and are secreted in the extracellular environment (therefore its disruption can lead to cancer) (MacDonald, 2009). Each member of the family has a number (Wtn1.Wtn2. Speaking of receptors and signalling pathways, Wnt proteins essentially perform a paracrine function by interacting with a complex receptor made up of a protein with seven transmembrane domains (FRIZZLED(Fz) of approximately 650 amino acids and a protein related to the LDL (Low-Density Lipoprotein) receptors and called" LRP" (LDL-RELATED PROTEIN) (Komiya and Habas, 2008).

Wnt assembles the dishevelled protein (Dsh or Dvl) on the extracellular side, and Fz and LRP recruit it on the intracellular side. Glycogen Synthetase Kinase 3 (GSK3) is a protein kinase that Dsh/Dvl recruits to phosphorylate the intracellular portion of LRP and to stop phosphorylating beta-catenin (approximately 1000 amino acids) (MacDonald and He, 2009). When beta-catenin is no longer phosphorylated, it can no longer be conjugated to ubiquitin or destroyed by the proteasome (Maurice, 2017). In

order to achieve this, the transcription factor beta-catenin can interact with other transcription factors, in particular TCF, to excite the Wnt target cell when it enters its nucleus (EN) (Qiuyu.Guo, 2021)

Nevertheless, the canonical Wnt pathway is required in the polarization of the anteroposterior axis in several animal species (Darras, 2018). Note that the new data obtained in the hemichordate saccoglossus question the regionalization model currently accepted in vertebrates(Aronowicz and Lowe, 2006).

1.7.1. TYPE OF WNT BETA-CATENIN SIGNAL PATHWAY

So much progress has been made in the last few decades in molecular medicine, which is currently experiencing rapid advancement. It has made the investigation process easier by making the use of molecular mechanisms possible. Wnt signalling pathways are a ground-breaking mechanism that exemplifies the fine art of science and diagnostic medicine. The Wnt is well known for controlling embryonic development as well as cell migration, proliferation, and fate determination.

Wingless and Int-1 combine to form the name Wnt (Komiya and Habas, 2008). The Wnt signal can be activated when the Wnt proteins interchange through the cysteine-rich N-terminal region of the frizzled receptor (Komiya and Habas, 2008).

The receptor can include the G-protein coupled receptors by resolving several times around the plasma membrane (Weinberg and Manojkumar, 2019).

However, co-receptores must engage with the Wnt protein and frizzled receptors in order for Wnt signalling to function to its maximum capacity. Signals are transmitted to the cytoplasm of cells with dishevelled phosphoproteins as a result of receptor activation, and signal transduction occurs between frizzled and dishevelled cells. Additionally, Dsh proteins are present in all organisms, and their functions in a Wnt pathway are classified into three main categories: canonical, non-canonical, and planar polar pathways (Komiya and Habas, 2008).

1.7.1.2.Canonical Pathway

As suggested by its name, the canonical route belongs to a group of canonical pathways that are connected to beta-catenin (b-catenin) (Komiya and Habas, 2008). b-

catenin may accumulate in the cytoplasm as a result. Induces the nucleus to translocate transcriptional factors and their coactivators. Instead of building up, b-catenin would be torn down by a destruction complex if Wnt were lacking. (Stamos and Weis, 2013). Additionally, several additional proteins that are in charge of destroying the b-catenin complex perform the role of the destruction complex.

These proteins (CK1a) are made up of several proteins, such as adenomatous polyposis Coli (APC), Glycogen Synthase 3 (GSK3), and Casein Kinase 1a (Stamos and William, 2013).

Ubiquitination, a process used by these proteins to break down b-catenin, can move it automatically to the proteasome for digestion (Szymanska, 2022). Additionally, Wnt binds to Fz and LRP5 after the digestion process and also inhibits the activity of the demolition compound (MacDonald, 2009). Axin can connect to LRP5 via the cytoplasmic tail after being phosphorylated in the destruction complex. The axin is then dephosphorylated, which lowers its stability level (Schaefer, 2020). The Dish is then phosphorylated, and the DIX and ODZ domains of GSK3 are used to block it as well as activate it. B-catenin can then build up and be stored in the nucleus as a result. However, the transcription factor and gene transduction inhibition of the cellular response (Duda and McCubrey, 2020). It is unknown how b-catenin activates target gene expression, and tissue-particular competitors appear to have the potential to help b-catenin define the target genes (Valenta and Basier, 2012). there are various unanswered problems regarding how the b-catenin protein interacts, including whether the Akt-induced phosphorylation of Ser552 causes the protein to become detached from cell-to-cell contact and accumulate in the cytosol (Fang and al., 2007). Non-canonical Pathway

LRP-5/6 is not used as a co-receptor in the non-canonical planar cell polarity (PCP) pathway, which lacks b-catenin (Ren and Liu 2021). When Wnt ties up to Fz and its co-receptor, the PCP pathway can be triggered. recruitments of Dsh leads to the formation of a compound with Dishevelled-associated activator morphogenesis and Dsh utilises its PDZ and DIX domains for the activation process (Masuko Katoh, 2017). DAAM1 then start up G-protein Rho through a guanine exchange factor, and Rho then activates Rho-associated kinase (ROCK) (Habas, 2003). An important cytoskeleton-regulating mechanism is called ROCK (Amano and al., 2010).

1.7.1.3.Non-canonical Wnt/calcium Pathway

Although it has no direct effect on calcium, b-catenin helps regulate calcium and calcium release from the endoplasmic, reticulum (ER) to control calcium at the intracellular level in this way. This pathway can be rationally explained by the same mechanism that controls other Wnts pathway, ligand interaction will encourage the direct activation of the Fz receptor , the Fz receptor will then connect with Dsh to activate Dsh protein domains (Komiya and Habas, 2008). signalling pathway involves both the PDZ and DEP domains (Komiya and Habas, 2008). Additionally, the Fz receptor exhibits a variety of properties that set it apart from other Wnt pathways. One such property is an indirect result of its interaction with trimeric G-protein (MacDonald, 2012). But when Dsh and G-protein are stimulated simultaneously, PLC and/or cGMP-specific PDE activation may result. A plasma membrane component's cleavage into DAG and IP3 can result from PLC activation (Cocco and Suh, 2015). IP3 binding to ER receptors can result in the release of DAG levels, and calcium can activate Cdc42 through PKC (Feng, Chiao, Tsai et al., 2015). When it comes to ventral patterning, Cdc42 is crucial. CaMKII prevents the activation of the transcription factor, which is a regulatory mechanism for cell adhesion, migration, and tissue separation. Calcium increases will activate both calcineurin and CaMKII (Wang and Zhe, 2015). However, a convergent Wnt pathway has been suggested after closely examining the connection of both canonical and non-canonical pathways (Komiya and Habas, 2008). The Wnt signalling pathway also shows integration and stimulation of the Wnt/Ca2+ and Wnt/b-catenin signalling for various other Wnt ligands, according to the convergent data (Komiya and Habas, 2008).

1.7.1.4. WNT/B-CATENIN ROLE IN BREAST CANCER

According to Shuang Shang, Flang Hua, and Zuo-Wei Hu (2017), the roles of b-catenin can be as an oncogene when it is translocated to the nucleus, attaches to T-cell factor or members of the lymphoid activating factor family, and transactivates its target genes.

1.8. THE GENE AXIN

Axin 1 and Axin 2, also known as Axin and conduction, respectively, the genes that code for the isoforms a and b, share 45 percent of their nucleotide identities and

functions. According to mice design study of Axin1 and Axin2 is expressed globally while Axin2 is expressed specifically expressed (Nicolac, Figeac, Peter, S.Zammit, 2015). According to (Stamos and Weis, 2013), Axin1 is an essential transcriptional factor component that aids in the degradation of catenin and controls the weak Wnt signalling activity (Stamos and Weis, 2013).

On the other hand, an increase in Axin2 occurs when b-catenin levels are high enough to function as a limiter of duration and amplitude of Wnt signals. Axin1 is dephosphorylated and activated in a Wnt-dependent way down-regulated (MacDonald, 2009).

When compared to the usual amount of Axin, the Axin that receives Wnt ligand signals has a lower concentration in the cell. According to biochemical studies, the intracellular concentration of Axin is 1000 times lower than that of other components of the demolition compound, indicating that Axin is the pathway's limiting element. (Salahshor and Woodgett, 2005).

45% of the nucleotides in the genes Axin 1 (Axin) and Axin 2 (conduction or Axil), which encode isoforms an b, are identical. According to Axin1 and Axin2 mouse model study, Axin1 is expressed generally, but Axin2 is expressed selectively. (Salahshor and Woodgett, 2005). Axin1 is a crucial transcriptional factor component that contributes to catenin degradation and is in charge of sustaining Wnt signaling activity's poor signaling (Thorvaldsen, 2017).

The duration and amplitude of Wnt signals can be constrained by Axin2 on the other hand when b-catenin levels are high. Axin1 is dephosphorylated and down-regulated in a Wnt-dependent way (MacDonald, 2009).

The concentration of axin, which receives wnt ligand signals, is lower in cell compared to the usual amount of axin. Axin is the limiting factor of the pathway, as evidenced by the fact that its intracellular concentration is 1000-fold lower than that of the other components of the demolition compound, according to a biochemical study (Salahshor and Woodgett, 2005).

1.8.1. AXIN1

After the murine-fused locus, axin1 was originally identified (Salahshor and Woodgett ,2005). The mouse protein and its human counterpart on chromosome 16p13.3 are 87% identical. While "isoform b" (GenBank NP 851393) is a condensed form of Axin in which 36 amino acids are lost in the N domain-terminal expressed by exon 8, it nevertheless encodes an 862 amino acid (aa) polypeptide . The unknown is the purpose of a polypeptide fragment encoded by exon 8. All species have this splicing variant, indicating that it has a comparable purpose (Thorvaldsen, 2017).

The polypeptide encoded by exon 8 is positioned betwixt the axin binding domain (DIX), the catenin binding domain and the dishevelled domain (Salahshor and Woodgett, 2005). The Axin oligomerization site, where Axin binds to itself, and a potential CKI phosphorylation site are both close by. Axin stability and function in cells are assumed to depend on axin dimerization. The spiced exon and two more potential nuclear export signals are sandwiched around a putative nuclear export signal for Axin (Salahshor and Woodgett, 2005).

1.8.1.2. AXIN2

Axin2 has been identified as a member of the Axin protein family as a result of its interactions with catenin. The colorectal mismatch repair system's catenin levels rise as a result of an Axin2 gene mutation (Salahshor and Woodgett, 2005). Numerous studies indicate that 11 exons cover more than 0.5 kb. Axin possesses two isoforms of Axin2, A and B, much like Axin1 did (Salahshor and Woodgett, 2005).

Axin2 was found to be localized In human chromosome 17q24, a place where the loss has occurred of heterozygosis is frequent in malignancies while breast cancer and neuroblastoma are two examples. APC, GSK3, and catenin binding domains, which are also present in Axin2, are additional domains (Salahshor and Woodgett, 2005).

Axin2 down-regulation has been linked to a worse overall survival rate in BC patients. The polymorphisms rs11079571 and rs393087 cause BC susceptibility (Salahshor and Woodgett, 2005).

1.8.1.3. AXIN VARIANT LINKED TO CARCINOMA

AXIN1 and AXIN2 are two variations that cause different types of AXIN, including breast cancer (Mazzoni and Fearon, 2014a). These genes' mutations are also domain-specific, affecting the catenin-binding domain and APC (RGS) among others (Mazzoni and Fearon, 2014a).

Numerous publications have been issued on the role of Axin 1 and Axin 2 mutations in cancers such as breast cancer, ovarian cancer, and HCC cell line. (Mazzoni and fearon, 2014b). AXIN2a expression has been shown to be significantly increased in breast cancer patients. In further studies, the expression of the genes for APC, b-catenin, CK1, GSK3, and PP2A was start to be associated with problematic clinical features (Sayad et al., 2021).

AXIN2 down-regulation is connected to worse overall survival in BC patients, according to a different study result (Salahshor and Woodgett, 2005).

1.8.1.4. AXIN LINEAGE WNT/B-CATENIN SIGNALING

Breast cancer has low expression of SOX7 and AXIN2 because Wnt/b-catenin signaling regulates their expression (Liu, Mastriani, 2016c). The SOXF gene family includes the tumor suppressor gene SOX7. Several types of human malignancies, including breast cancer, have been associated with this gene. Although their mode The mode of action is uncertain, prior research has been done to evaluate and examine the connection. Analysis of SOX7 and other co-expressed genes of the wnt/b-catenin pathway,performed to investigate the relationship among AXIN2 and SOX7 and their co-regulatory roles on the WNT/b-catenin signalling pathway was effective and revealed that the expression of SOX7, SOX17, and SOX18 was significantly lower than that of AXIN2. compared to healthy controls, in breast cancer tissue. According to the underlying study, AXIN2 and SOX7 have favorable interactions with one another in the WNT/b-catenin signaling pathway (Huidi Liu, Emilio Mastriani 2016). In clinicopathologic investigations, SOX7 down-regulation has been linked to advanced, poorly differentiated breast cancer. Through analysis and investigation using bioinformatics, SMAD7 was discovered to be a target of SOX7 and AXIN2 in the Wnt/b-catenin signalling pathway, that influences breast cancer cell development. SOX7 shows a positive association with AXIN but a negative correlation with catenin,

suggesting that SOX7 and AXIN2 have a co-regulatory function in the Wnt/b-catenin pathway.(Liu 2016a).

1.9. THE GSK3B GENE

In rat skeletal muscle, glycogen synthase kinase 3 (GSK3), a serine-threonine kinase, was discovered and identified in 1980. (MacAulay and Woodgett, 2008).

In addition, it is the sole enzyme that has been identified as phosphorylating glycogen synthase, as opposed to the GSK1 and GSK2 enzymes were previously known.

GSK3 has been associated with a number of biological processes including neurodegeneration, wound healing, immunological responses, apoptosis, autophagy, and carcinogenesis (Glibo, 2021). There are two different forms of GSK3: In humans, GSK3 and GSK3 isoforms are located on chromosomes 19q13.2 (GSK3) and 3q13.3 (GSK3) and share 85% of their DNAwith each other (Martelli, 2022). They both feature an alpha helix, a brief connecting section, and seven antiparallel plates, although they are encoded by different genes and manifest themselves in different ways in human tissues. (Martelli and McCubrey, 2022).

Despite their near resemblance and functional overlap, these isoforms are neither redundant nor functionally equivalent (Castellano and Santos, 2011). GSK3 is produced in natural killer (NK) cells, granulocytes of the borne marrow and ovaries by the GSK3B gene on the long arm of chromosome 3. since it lacks a rich domain, the signalling mechanism and protein function of GSK3 are relatively well characterised (Cichocki, 2017).

It is vital to remember that the GSK3B gene is fatal throughout embryonic development and that the production of one isoform cannot in any way make up for the loss of another isoform located both in the nucleus and in the cytoplasm (Glibo, 2021).

Dysregulation of GSK3B has been linked to both carcinogenesis and cancer recurrence. Breast cancer carcinogenesis appears to be negatively affected by GSK3B, suggesting that tissue-specific synthesis of a kinase-inactive (dominant negative) GSK3 promotes breast cancer by sinking endogenous GSK3 activity (Jia, Luo, 2009).

The acceleration of breast carcinogenesis by this inactive GSK3 kinase, along with the increase in catenin and cyclin D1, indicates that stimulation occurs by blocking the Wnt/b-catenin pathway (Wang, 2016). control of GSK3 by adiponectin in MDA-MB-231 human breast cancer cells leads to apoptosis and cell cycle arrest, which associated with reduced heights of cyclin D1 and reduction in the intracellular accretion of-catenin and its nuclear activities. GSK3 activation, on the other hand, reduces breast cancer progression (Wang, 2016).

Furthermore, administration of recombinant adiponectin or adnovirus-mediated adiponectin overexpression in nude mice dramatically reduced the mammary tumourigenicity of MDA-MB-231 cells when GSK3 was activated in vivo (Wang, 2016). Activation of GSK3 by rapamycin leads to cell cycle arrest, growth-dependent ancghorage inhibition and down-regulatio of cyclin D1 expression in breast cancer cells (160 since constitutively active GSK3 (S9A mutant) promotes apoptosis in human breast cancer cells, injection of GSK3-containing liposome complex into tumour-bearing mice significantly delayed breast tumour growth (Dong, 2005). Inhibition of GSK3 activity in mammary epithelial cells induces cancer but acts as tumour suppressor in mammary tumours (McCubrey, 2014).

1.10. MOLECULAR ANALYSIS OF APC AND ITS RELATION TO CARCINOMAS

The large protein with amino acids is encoded by an mRNA of over 9.5 kilobytes and is translated primarily by the APC gene found in the 5q21 region of the chromosome (Zhang and Shay, 2017).

There are 15 exons in the coding area, with the final one accounting for more than three-quarters of the whole. Both familial and sporadic colorectal cancer has been related to mutated APC genes, a tumor suppressor gene; somatic mutation of this gene also manifests extra colonic malignancy in the esophagus, pancreatic, and stomach cancer (Kwong, 2009).

In contrast to gastrointestinal tract carcinomas, initial breast cancer in males has extremely few APC gene alterations (Furuuchi, 2000). The importance of APC gene in primary breast cancer is supported and experimentally explained by a study in female mice, which points a squash mutation in codon 850 of this APC gene, which is

primarily responsible for the development of breast carcinomas. (Furuuchi, 2000). It is strongly advised against cancer. The APC gene, like in familial adenomatous polyposis, has a truncation mutation in its N-terminal region. (FAP), which affects more than 60% of the area of this gene. This mutation is located at exon 15 in the small region (MCR), which effectively represents 10% of the coding region (Leoz, 2015). Due to the vast variety of many mutations of the APC gene, the molecular genetic diagnosis of this gene is challenging for this purpose (Leoz, 2015). Nucleic acid-based methods for detecting APC mutations take a long time since more than 95% of APC gene mutations result in truncated APC proteins related to a number of defects such as frameshift, nonsense, and splice site mutations (Aitchison, 2020). Another possible tool for detecting APC changes is the protein truncation assay, which looks for truncation mutations in proteins in yeast or in vitro. (Futuuchi et al., 2000). Disruption of the adenomous polyposis coli (APC)/catenin pathway is associated with the development of breast cancer, and similar disruption is suspected for colorectal cancer. Since somatic APC and catenin mutations are rare in breast cancer, researchers are studying the effects of APC gene promoter methylation and the different mutations of the APC and catenin genes to better understand their involvement in primary breast cancer and non-melanoma skin cancer.While none were found in the 21 non-cancerous breast tissue, they discovered 18 out of 50 CpG island hyper methylation of the APC promoter in the main breast tumor (Chang, 2016).

Notwithstanding the relevance of APC and catenin mutations, no association was found between APC promoter hypermethylation and a variety of parameters, counting patient age, lymph node status, metastasis, existence or truancy of oestrogen and progesterone receptors, tumour magnitude, phase, or histological type. (Feng 2018d). CpG island hypermethylation in the APC promoter, is,to our knowledge, a cancer-specific modification that was previously thought to be a general mechanism in APC gene silencing in primary breast cancer. (Brooks and Zeleniuch, 2009).

1.11. THE NANOBUBBLE OZONE STORED NIOSOMES(NOSN)

A noisome that contains nanobubble ozone is a colorless gas with three oxygen atoms in its composition (Molina and Sherwood, 1985). The ozone gas layer shields the globe from the sun's ultraviolet radiation. Ozone is, in fact, the hazardous atmospheric pollutant closest to the earth. When people breathe ozone, their throats and lungs get

irritated, they cough more, and their asthma symptoms worsen (Manisalidis.,Stavropoulo., and Stravopoulos., 2020).

nanobubble ozone possesses therapeutic qualities that might be utilized to treat cancer, ischemic heart disease, rheumatoid arthritis, viral infections like HIV and SARS, wound sterilization, and even viral infections like human immunodeficienty virus (HIV) and SARS (Clavo and Esparragon, 2018).

Since many years ago, oxidative stress has been produced using ozone (O3) and other forms of ionizing radiation, changing molecular pathways, and slowing the development of tumor cells without hurting healthy cells (Mehdi, Sharifi-Rad, 2020). The strong relationship between tumor cell proliferation and ozone-specific ROS concentration suggests that raising ozone concentration efficiently inhibits tumor cell development (Liou and Storz, 2010e).

an experiment was carried out in which the breast cancer cell line in humans, uterus, and lung tumour was treated for 8 days in culture with differents concentrations of ozone from 0.3 to 0.8 parts per million, the results showing that 0.3 to 0.5 parts per million inhibited the progress of the cancer cells by 40-90%. (Clavo and Esparragon, 2018).

1.11.1. THE RECENT USE OF THE NANOBUBBLE OZONE STORED NIOSOMES (NOSN)IN THE THERAPY FOR THE TARGET TUMOR THERAPIES.

Radiation treatment (RT) and chemotherapy (CT), two common surgical removal techniques for cancer, continue to be successful (Baskar, 2012).

Innovative cancer medicines, however, such immunotherapy and reactive oxygen species (ROS) therapy, are very promising (Ruolan Liu 2022). Some RT and CT drugs cause the production of reactive oxygen species (ROS) and free radicals in cancer cells, which generally damage cancer cells (Aggarwal, 2019b).

Because of the well-known Warburg effect (aerobic glycolysis or aerobic fermentation of glucose), cancer cells have a poor tolerance to the increased creation of oxygen

containing reactive species (ROS) (Asare-Werehene, 2019). ROS produced outside might throw the system's equilibrium off. It should be emphasized that there is evidence linking the Warburg effect, extremely high glucose intake, and the cell's ability to undergo apoptosis to enhance the generation of oxygen- containing reactive specieies(ROS) in cancer cells (Liou and Storz, 2010). On the other hand, the body generates adaptation by manufacturing more antioxidant enzymes and improving specific metabolic pathways (for example, the pentose phosphate pathway of glycolysis produces NADPH), which contributes in the reduction of excessive oxygen containing reactive species(ROS). (Mullarky and Cantley, 2015).

The secondary effect, however, could be crucial in oncogene-induced senescence, according to recent research (OIS) (Quijana, 2012). Tumor shrinkage is the biological role of oncongene induced senescence (OIS); furthermore, oncogene induced senescence (OIS) is modulated by the oxidation of glucose caused by pyruvate dehydrogenase (PDH). ROS have been linked to cancer because they improve cell motility, survival, and proliferation (Kamarajugadda, 2012b). Tyrosine phosphatases and the inactivation of tensin homologs produce chromosomal damage, which leads to tumorigenicity and tumour formation.oxygen containing reactive species (ROS), on the other hand, can disrupt cell membranes and have other undesirable repercussions, which can lead to cell damage. (Liou and Storz, 2010e).

CHAPTER 2: MATERIALS AND METHODS

2.1 EXPERIMENTAL ENVIRONMENT

This study was conducted at DESAM Resarch institute LaboratoryNear east University in North Cyprus, Nicosia, Cell-culture Laboratory was used for cell culture experiments and NOSN treatment. RNA isolation, cDNA synthesis and qPCR experiments were conducted in molecular genetics laboratories.

2.1.1 MATERIALS

2.1.2. BIOLOGICAL MATERIAL

MDA-231-MB cell line, was used,which was a kind gift from Prof. Dr Pinar Tulay (MD Anderson; pleural effusion from a patient with invasive ductal carcinoma and is frequently used to mimic metastatic breast cance).

2.1.3.CHEMICAL MATERIALS

Fetal Bovine serum(FBS) (Ref 10500064, Penicillin, Streptomycin, and Thermofischer scientific, pittsburg, USA (Ref 15140122, thermofischer scientific, pittsbug, USA), Dulbecco modified eagle medium (DMEM) F-12, and HEPES (Ref 3133008, thermofischer scientific, Pittsburg, USA) were all used during the test. (Patent No,PCT/TR2022/050177, Sonofarma pharmaceuticals chemical industry trade Ltd Sti), Nanobubble ozone stored niosomes (NOSN). TRYSIN/EDTA (0.25%) (Ref 25200056, during our cell culture experiment, the linked cells in the flask wre separated using this apparatus (produced by Thermofischer Scientific, Pittsburgh, USA), resulting in cells overlaid on the the surface and nanobubble ozone stored niosomes (NOSN) (patent N°,PCT/TR2022/050177, Sonofarma pharmaceuticals chemical industry trade Ltd Sti) were used during cell culture experiments.

For RNA isolation, the Trisol reagent (Hibrigent,based in Istanbul, Turkey), and the ABM one script plus cDNA-synthesis kit (ABM, Richmond, Canada) were employed 2X SYBER Green Qpcr mix (Hibrigen, from Istanbul in Turkey) were used for Qpcr experiments.

2.1.4.CELL LINE

MDA-MB-231 (ATCC#HTB-26) was used for the cell culture experiments, which were kindly provided by Prof Dr Pinar Tulay. MDA-MB-231 cells are epithelial cells isolated from the breast tissue of a white 51-year-old female, patient with adenocarcinoma. Detailed information about the characteristics of cell line can be found in the introduction section of this thesis.

2.1.5. LABORATORY MATERIALS OR INSTRUMENTS

- Metisafe laminar air flow cabinet from Ankara in Turkey
- Sanyo MCO-5ac Incusafe compact CO2 Incubator from Osaka Japan
- Metisafe PCR cabinet from Ankara in Turkey
- Nano-drop 2000/2000c Spectrophotomer (Thermo-scientific Pittsburg in USA
- RotorGene Real-Time PCR (Qiagen, Hilden in Germany

2.1.6. OLIGONUCLEOTIDES

The Turkish Oligomer company provided the primers used in the experiments.

2.2. METHODS

2.2.1. PREPARING MDA-MB-231-CULTURE MEDIUM

Culture medium was made with DMEM/F-12(1.1) (1X) (Delbecco modified eagle medium F-12+1+L-Glutamate,+15 Mm HEPES 10% FBS (fetal bovine serum) and 1% pen-strep (penicillin and streptomycin).

2.2.2. THAWING CELLS

MDA-MB-231 cells were kept frozen at -80 °C using cryotubes containing 5% DMSO (Dimethyl Sulfoxide): DMSO helps to prevent the production of ice crystals.

Once melt, cells were transferred to falcon tube containing 15ml of culture media. They were centrifuged at 22° 1000 RPM 192, supernatant was discarded, pellet was dissolved with 5ml culture media and transferred to T25 culture flask.

2.2.3. CELL CULTURE

cells were incubated for 24 hours at 35°C, 5 CO2 incubator.

2.2.4. MICROSCOPIC OBSERVATION

cells were visualised under microscope to evaluate morphology and confluency.

2.2.5. NANOBUBBLE OZONE STORED NIOSOMES (NOSN) TREATMENT

MDA-MB-231 cells were treated with different concentrations of NOSN, as represented in the table below One vial served as an untreated control. The samples were then incubated for 24 hours at 37°C in a 5% CO2 incubator.

Table 2.2.5.1 Ozone concentrations that were applied to MDA-MB-231 cells.

Flask	Ozone concentrations (ppm)
1	6.25 ppm
2	3.125 ppm
3	1.5625 ppm
Control	No ozone

2.2.6. CELL PELLET FORMATION

Growth medium from with the NOSN was removed from flasks 1ml of trypsin was added to make the cells split at the bottom, and then incubated the flask for 5 minutes to get the cells layered on the surface. The flask media containing the cells and culture were combined with 3ml of fresh growth medium after the incubation period, and the mixture was then centrifuged for 5 minutes at 13000 rpm. The supernatant was saved after centrifugation, and the pellet was kept at -80°C for further RNA extraction.

2.2.7. RNA ISOLATION

The first step in an RNA extraction procedure is to suspend the cell pellet in 500 ul of Trizol solution. The samples are then vortexed for 15 seconds, allowed to stand at room temperature for 2-3 minutes and then centrifuged for 15 minutes at 8°C at no more than 12,000 rpm. The mixture separates into a lower red phase, a phenolchloroform phase, an interphase, and an upper aqueous phase without color following the subsequent centrifugation. The aqueous phase at the top is then carefully transferred into a fresh tube, ensuring that the RNA is the only component present

there. Then, 250ul of isopropyl alcohol was used to precipitate the RNA out of the aqueous phase. After being incubated for 20 minutes at 15°C to 30°C, the samples were centrifuged at a maximum speed of 12,000 rpm for 10 minutes at 4°C. as the RNA precipitate, which is occasionally invisible before spinning, forms a gel-like pellet on the side and bottom of the tube, we completely removed the supernatant after centrifigatuin. The RNA pellet should then be mixed with 500 µl. of 75% ethanol and washed once. Vortex the samples to combine them, then centrifuge at no more than 1000 rpm for 5 minutes. We must air-dry the RNA pellet for 5 to 10 minutes after removing all of the ethanol left over from the previous washing procedure. We added 30 µl. of Dnase-and Rnase-free water to elute the RNA.

2.2.8. COMPLEMENTARY DNA (cDNA)

Following RNA isolation, complementary DNA (cDNA), were synthesized with ABM one Script Plus cDNA synthesis kit. Reaction mix for each sample included 1ul of oligo (dT) primer, 1ul of deoxyribonucleotides triphosphate (dNTPs), and 3ul of water for component preparation. Using a mini-centrifuge, we centrifuged our component for 15 minutes before incubating the tubes. By heating the sample to 55°C for 15 minutes,in a conventional PCR machine allow us to automate reverse transcription and produce cDNA from RNA samples.

2.2.9. REAL TIME (QUANTITATIVE PCR)

RNA expression patterns of the *APC,AXIN*, *BETA-CATENIN*, and *GSK3B* genes after applying nanobubble ozone stored niosomes to the breast cancer cell line MDA-MB-231,was investigated using gene specific primers, BETA-ACTIN(ACTB) was used as a housekeeping gene for normalization (Qiagen) instrument and 2X SYBER Green qPCR Mix (Hibrigen, Istanbul, Turkey) was used for qPCR expression .The qPCR master mix calculations are shown for each gene in Table 2.2. because and Table 2.3 and Table 2.4 show the primer sequences, melting temperatures, GC content, and predicted product sizes for each gene. Because we did not acquire any data for *GSK3B* (no amplification), we used the criteria given in Table 2.5 below.

A no-template control was added in each RT-qPCR process to screen for potential contamination. Following the completion of the real-time PCR experiment, the cycle threshold was automatically modified and C-values were computed.

TABLE.2.2.9.1 this table show us the RT-qPCR mixture calculation for *APC*, *AXIN*, β-catenin and *ACTB*

COMPONEBTS	FINAL CONCENTRATION	MIXTURE VOLUME FOR 1X
SYBR,Mix	1X	10 µl.
FORWARD Primer	0.9 µM	2 µl.
REVERSE Primer	0,9 µM	2 µl.
dH2O	0,9 µM	5 µl.
Cdna	0,9 µM	2 µl.
Glycerol (For GSK3B and β-catenin)	4,5%	1 µl.

TABLE.2.2.9.2. this table show us the RT-Qpcr mixture caculation for GSK3

COMPONENTS	Final Concentration	Mixture volume for 1X
SYBR Mix	1X	10 µl.
Forward primer	0,8 µM	2 µl.
Reverse primer	0,8 µM	2 µl.
dH2O	0,8 µM	6 µl.
Cdna	0,8 µM	2 µl.
Glycerol (for GSK3 and β-catenin	4,3%	1 µl.

TABLE.2.2.9.3. this table show us the Real-Time PCR Conditions for β-Actine

	PCR Steps	Temperature °C/Time	cycles

Steps	**Initial Denaturation**	95°C/ 5 minutes	1
	Denaturation	95°C/ 0.30 seconds	30
	Annealing	60°C/ 0.30 seconds	
	Elongation	72°C/ 0.45 seconds	
	Termination	72°C/ 7 minutes	1

TABLE.2.2.9.4. this table show us the Real-Time PCR Conditions for β-catenin and GSK3B

	PCR Steps	**Temperature °C/Time**	**Cycles**
Steps	**Initial Denaturation**	95°C/ 2 minutes	1
	Denaturation	95°C/ 0.30 seconds	30
	Annealing	59°C/ 0.30 seconds	
	Elongation	72°C/ 0.45 seconds	
	Termination	72°C/ 10 minutes	1

TABLE.2.2.9.5. this table show us the Real-Time PCR Conditions for APC, AXIN

	PCR Steps	**Temperature °C/Time**	**Cycles**
	Initial Denaturation	95°C/ 2 minutes	1
	Denaturation	95°C/ 0.30 seconds	

Steps	**Annealing**	58°C/ 0.30 seconds	30
	Elongation	72°C/ 0.45 seconds	
	Termination	72°C/ 7 minutes	1

2.10. STATISTICAL ANALYSIS

SPSS software (statistical package for the social sciences 2.5.0, SPSS inc) was used to conduct the statistical analysis. The information was displayed as mean standard erro (SE). Gene expression data were gathered using the cycle threshold approach (CT=Cycle number at which logarithmic PCR graphs cross a predetermined threshold line). The expression of each gene between the deposits was compared using the CT formula (CT=CT of the target gene CT of the housekeeping gene). Differences between normal and paradoxical allocate factors were analyzed using the Kruskal-Wallis . P<005 indicates statistical significance.

CHAPTER 3: RESULTS

3.1. RESULTS

As explained above, MDA-231-MB cells were treated different concentrations of nanobubble ozone stored niosomes (NOSN) and a control group without traitmentwas involved . Upon treatment, RNA isolation and cDNA synthesis was done for each group. Expression of *APC, BETA-CATENIN, AXIN, GSK3B* and housekeeping gene *ACTB* were done with gene-specific primers and ΔΔCT calculations were done to determine the fold change in gene expression levels in arbitrary units for each gene in each group compared to the control group. This chapter contains results from the experiments.

Table 3.1.1. Show us the ct values of the APC, β-CATENIN ,AXIN, GSK3B.

APC	APC	APC	APC
Sample	CT.1	CT.2	CT.MEAN
Control	24.03	23.96	23.995
1	20.83	20.47	20.65
2	21.88	21.73	21.805
3	24.62	22.66	23.64

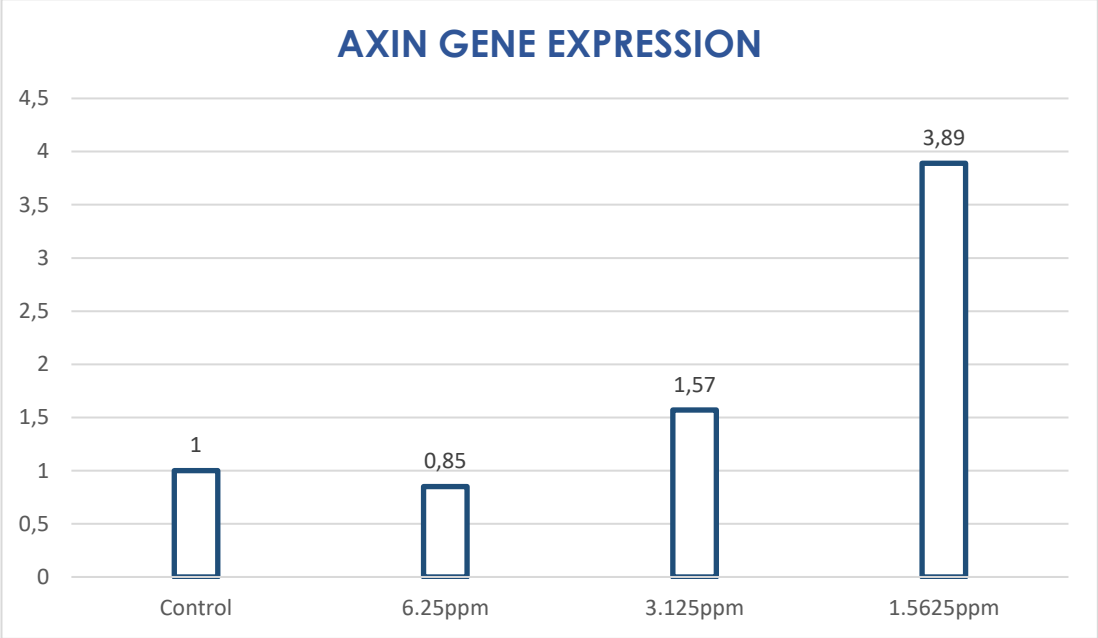
	B-CATENIN	*B-CATENIN*	*B-CATENIN*
SAMPLE	CT.1	CT.2	CT MEAN
CONTROL	22.84	21.48	22.16
1	19.76	19.69	19.725
2	22.52	19.29	20.905
3	17.79	18.11	17.95

AXIN	AXIN	AXIN	AXIN
SAMPLE	CT.1	CT.2	CT.MEAN
CONTROL	20.97	20.49	20.73
1	22.42	19.77	21.095
2	18.28	20.65	19.465
3	19.85	22.53	21.19

GSK3B	CT.1	CT.2	CT.MEAN
SAMPLE	NO EXPRESSION		
CONTROL			
1			
2			
3			

3.2. *AXIN* GENE EXPRESSION

Changes in mRNA expression levels of AXIN gene were investigated in MDA-MB-231 triple negative breast cancer cells that were treated with different concentrations of NOSN, as explained above. Upon normalization with the expression of a housekeeping gene (ACTB), fold changes were calculated by comparing three groups treated with different NOSN concentrations with control group. Fold change graph is represented in Figure 3.2.1. In the graph, *Y-axis* represents the fold changein arbitrary units and groups with different NOSN concentrations are represented in the *X-axis* of the grap



Nanobubble ozone stored niosomes

Figure 3.2.1: The statistical analysis of the log ΔΔCT of value AXIN in different concentration nanobubble ozone stored niosomes (*p<0.005)

3.2.2. APC GENE EXPRESSION

Changes in mRNA aspect points of *APC* gene were studied in MDA-MB-231 triple negative breast cancer cells that were preserved with diverse focuses of NOSN, as enlightened above. Upon regularization with the countenance of a housekeeping gene (ACTB), fold changes were considered by relating three groups cured with different NOSN concentrations with control group. Fold change graph is personified in Figure 3.2.2.1. In the graph, *Y-axis* embodies the fold changein arbitrary units and groups with different NOSN attentions are symbolized in the *X-axis* of the graph.

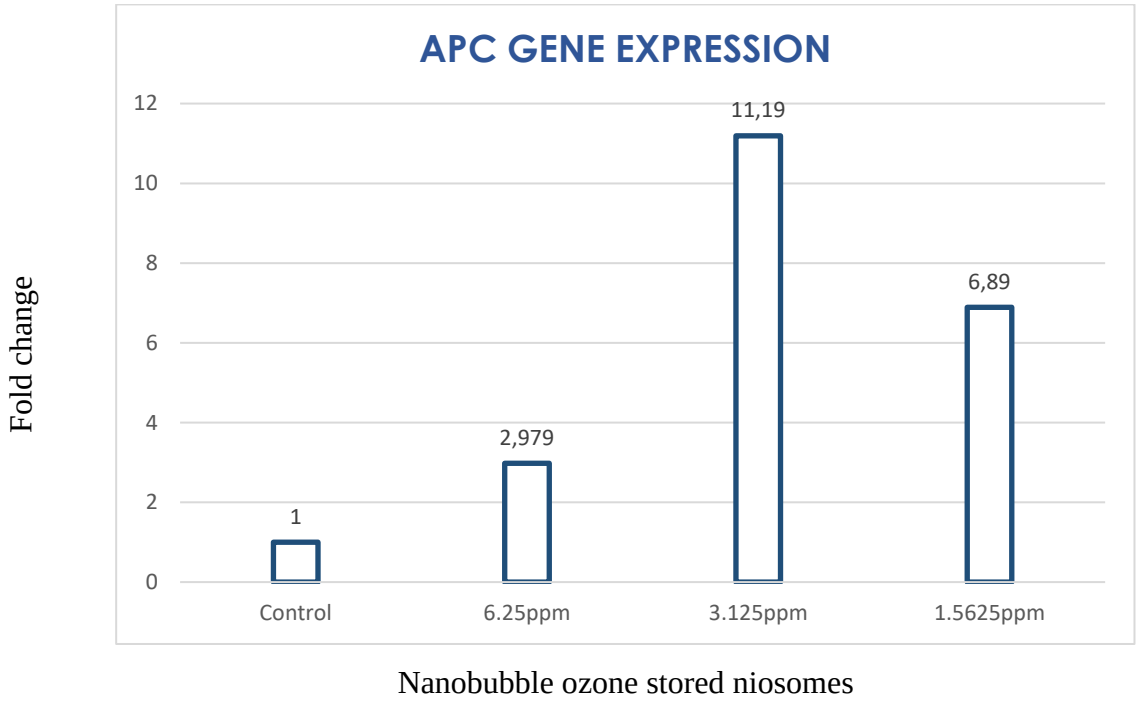


Figure 3.2.2.1: The statistical analysis of the log ΔΔCT of value *APC* in different concentration nanobubble ozone stored niosomes (*p<0.005)

3.2.3. B-CATENIN GENE EXPRESSION

Changes in mRNA utterance stages of *B-CATENIN* gene were scrutinized in MDA-MB-231 triple negative breast cancer cells that were cured with dissimilar attentions of NOSN, as clarified above. Upon standardization with the utterance of a housekeeping gene (ACTB), fold changes were intended by balancing three groups cured with different NOSN concentrations with control group. Fold change graph is embodied in Figure 3.2.3.1. In the graph, *Y-axis* symbolizes the fold changein arbitrary units and groups with different NOSN concentrations are illustrated in the *X-axis* of the graph.

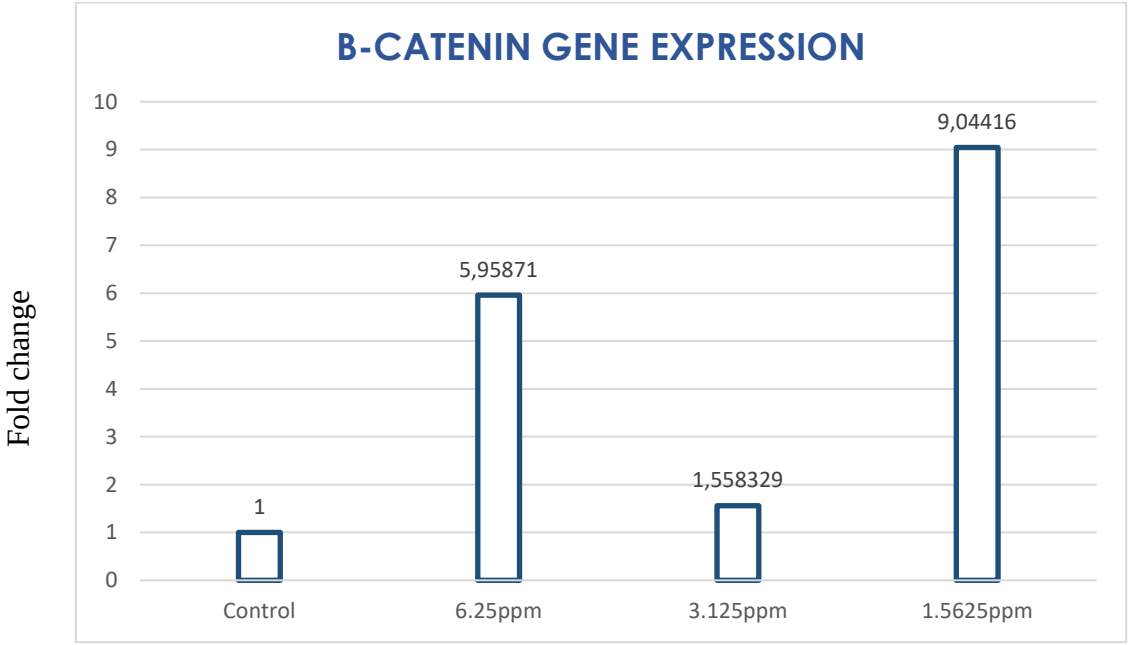


Figure 3.2.3.1: The statistical analysis of the log ΔΔCT of value *B-CATENIN* in different concentration nanobubble ozone stored niosomes (*p<0.005)

CHAPTER 4: DISCUSSION AND CONCLUSION

4.1 DISCUSSION

Breast cancer is demarcated as a compilation of clear malignancies that obvious in the mammary glands (Feng, 2018a). carcinomas constitute the most of breast cancer stint sarcomas such as phyllodes tumors and angiosarcomas are scarcely seen (Feng, 2018a). Cancer starts when healthy cells in the breast cancer swich and grow out of sway, forming a mass or sheetcalled tumor of cells (Cooper M. Geoffrey, 2000).

Breast cancer is the greatest frequently detected cancer in women wordwide with 2026 million (95% UL, 2.24-2.79 million) new cases in 2020 (Lukasiewicz, 2021b). The greatest common source of hereditary breast cancer in an inherited mutation in breast cancer gene 1 (BRCA1) or breast cancer gene 2 (BRCA2), women with a breast cancer gene 1 (BRCA1) mutation a 55-65% lifespan probability of starting breast cancer (Feng, 2018a). for a woman with breast cancer gene 2 (BRCA2) mutation the lifetime risk is 45%. A woman with breast cancer gene 1 (BRCA1) or breast cancer gene 2 (BRCA2) has all over 70% chance of developing breast cancer by age 80 (Feng 2018 a). The effect of mutation depends on how several other family members have breast cancer the likelihood increases the more family members are affeted (Feng, 2018a).

Breast cancer risk may be increased by contemporary lifestyle factors such as excessive alcohol consumption an smoking, as alcohol consumption can stimulate oestrogrn receptor pathways and blood levels of oestrogen-associated hormones (Sun et ., 2017). Women who smoke and dring are also at higher risk of developing breast cancer, as mutagens from cigarette smoke have been found in the breast fluid of non-breastfeeding women (Sheng et al., 2017). Long-term survival rates will increase with knowledge about the nature of breast cancer recurrences (Lukasiewicz, 2021b). The researchers of the European union-funded project 3D breast cancer (Miodelling breast cancer in 3D cell culture systems and animals) have addressed this problem by exploring the mechanisms behind tumour theory, cell escape and cell survival (Leonard and Godin, 2016). A new method for researching tumor-initiating cells and understanding the underlying mechanisms of relapse has been made possible by the 3D breast cancer culture system (Bushenell, 2021). It should be noted that cell culture

is an experimental approach that has been widely used since beginning of cancer reaserch. This method has contributed a significant amount of knowledge about the biology of cancer cells and is still frequently a necessary step, there are additional types of cultures, such as diploid primary, secondary, or continuous cell lines (Mirabelli, 2009). Over 90% of the MDA-MB-231 breast cancer cell line population has low CD44+/CD24 content and is a triple negative cell line with a mesenchymal phenotype (T.Hero, 2019). shows typical epithelial mesenchymal transition (EMT) associated with the development of cancer, there is no by desceiptions of the structure and biological traits of MDA-MB-231 spheroids, that cells further study (Huang, 2020). MDA-MB-231 breast cancer cells were inserted into the fourth mammary fat pad and described as a pratical guide for an orthopaedic example of triple negative breast cancer that closely mimics the behaviour of cancer cells in humans (S. Cheng, 2020).

Angiogenesis in cancer is essential for tumor growth and spread and is initiated by chemical signals from rapidly developing tumor cells (Folkaman, 1971). Angiogenesis is triggered when the tumor tissues want nutrition and oxygen, angiogenesis is controlled by activator and inhibitor molecules (Nayo.Nishida, 2006). When new blood vessels are visible, angiogenesis can be natural, healthy biological function (Tahergorabi and Khazaei, 2012). It happens in the context of child growth when the endometrium is emptied in women during menstruation each month and new blood vessels are needed for healing (Critchley, 2020). Angiogenis play the main part in the formation of new blood vessels and in the metastasis of malignancies diseases (Lugano, 2020a).

Wnt/b-catenin is a protein whose gene is CTNNB it plays role in cell adhesion, cell signaling, and gene transcription (MacDonald, 2009). In addition, it comprises a central part made of repeated amino acid units, a C-terminal part constituting an alpha helix and an N-terminal part (S. Legardinier, 2009). It should be noted that the latter acts as a transcriptional coactivator in the Wnt pathway, it uses this pathway, regulates angiogenesis in the central nervous system, and participates in the formation of the blood-brain barrier (K. Hübner, 2018). It is also, with cadherin with which it binds, and alpha-catenin, a constituent of adherens junctions and therefore plays an important role in intercellular adhesion, particularly at the neuronal level (Mège, 2017). It intervenes in the remodeling of synapses and thus plays a role in memory. At rest, b-

catenin is phosphorylated by GSK3B, which marks it for degradation for the proteasome (Mège, 2017). In medicine, certain mutations of CTNNB1 are associated with an intellectual deficit, one of these mutations induces an autosomal dominant syndrome associated with hypotonia during childhood, spasticity of the lower limbs, disorders of higher functions, and abnormalities of the facies (Ho.KL and Chung, 2022). The large protein of 2843 amino acids is encoded by an mRNA over 9.5 kilobytes in size and translated mainly by the APC gene located in region 5q21 of the chromosome (Wang, 2019). The importance of this APC gene in primary brast cancer is supported and experimentally explained by a study in female mice which points to a nonsense mutation in codon 850 of this APC gene, that is primarly accountable for the development of breast carcinomas (Furuuchi, 2000). Due to the great variety of many mutations of the APC gene, the molecular genetic diagnosis of this gene challenging (Leoz, 2015). Axin1 and Axin2 are two variants that cause different forms of Axin, including breast cancer because Wnt/b-catin pathway genes that was conducted to study the relationship between AXIN2 and SOX7 and their co-regulated roles on the Wnt/b-catenin signaling pathway which was conducted to study in the relationship betwixt SOX7 and AXIN2, SOX17, and SOX18 expression were all significantly lower than that of AXIN2 (Dai et al 2017). According to the underlying study, AXIN2 and SOX7 have favorable interactions with one another in the Wnt/b-catenin signaling pathway (Liu, 2016b). The gene GSK3B in rat skeletal muscle the glycogen synthese kinase 3 (GSK3) serine-threonine kinase was discovered and identified in 1980 (Geetha Vani Rayasan, 2009). In humains there are two distincts forms of GSK3, GSK3 isoforms are located on chromosomes 19q13.2 (GSK3) and share 85% of their DNA between them (Martell,i 2022).

The nanobubble ozone stored niosomes is a colorless gas with three oxygen atoms in its composition (Molina and Sherwood, 1985). nanobubble ozone possesses therapeutic qualities that might be utilized to treat cancer, ischemic heart disease, rheumatoid arthritis, viral infections like HIV and SARS, wound sterilization, and even viral infections like human immunodeficienty virus (HIV) and SARS (Clavo and Esparragon, 2018).

The recent use of nanobubble ozone stored niosomes was for radiation treatment and chemotherapy two common surgical removal techniques for cancer continue to be

succeful (Baskar, 2012). Innovative cancer medicines, however, such immunotherapy and reactive oxygen species (ROS) therapy, are very promising (Liu, 2022a). Finaly we have complex statements for the AXIN,APC,GSK3, and Catenin genes. The four gene's descriptions provide evidence that assiduous ozone has abolished their RNA expression, they also show improved expression in the time. The production of catenin is decreased by nanobubble ozone, which inhibits the Wnt signaling pathway, a crucial one for the development and spread of cancer.

4.2. CONCLUSION

The goal of this study is to determine how the beta-catenin pathway genes in the MDA-231-MB breast cancer cell line are impacted by nanobubble ozone stored niosomes. These four disting gene sets (*axin,apc,gsk3b* and *catenin* genes) are acquired interchangeably as beta-catenin pathway genes and work in tandem with the CK1 gene to regulate the Wnt/b-catenin signaling pathway,*AXIN,APC,GSK3,* and CK1 make form the delicate abolition compound. This demolition composite rigorously resolved the amount of beta-catenin because its unwavering focus severely damaged the protein.

AXIN,GSK3,APC, and *CK1* make formthe complex which is fragile. The amount of beta-catenin was meticulously resolved since the destruction complex's unwavering emphasis severely harmed beta-catenin. This lessens beta-catenin's impunity since beta-catenin regulates the Wnt/b-catenin pathway's activation, which encourages the formation of cancer stem cells (CSCs) and subsequently causes cancer metastasis and degradation.

In order to control whether the expression of these complex destruction genes deviated from their typical expression, we mainly used various volumes of nanobubble ozone stored niosomes in our investigation. Destructive genes will express themselves more, which logically implies that they will continue to break down beta-catenin. Because b-catenin only partially activates the Wnt signaling pathway, cancer metastasis may be prevented.

We are unable to conclusively say that the fluid absorption of nanobubble ozone stored niosomes will be related to the treatment of breast cancer by examining gene expression at the mRNA amount, and we have a limit of nanonobubble variability. However, the conformation will be made in the time ahead taking into account the greater variability of nanobubble ozone stored niosomes.

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