

EXPRESSION ANALYSIS OF B4-GALT-III & B4-GALT-IV IN BREAST CANCER

PROJECT WORK

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

By

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**DEPARTMENT OF BIOTECHNOLOGY
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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
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This is to certify that this Project Report is the bonafide work of **Nithya Sri. G (40230031)** who carried out Project work entitled "**EXPRESSION ANALYSIS OF THE GENES B-4GALT-III & B-4GALT-IV IN BREAST CANCER**" under my supervision from October 2023 to March 2024.

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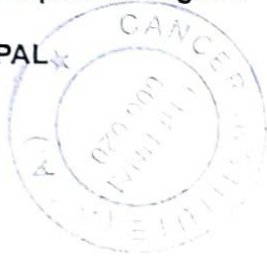
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This is to certify that, **Nithya Sri. G** carried out this dissertation work entitled "**Expression analysis of B4GALT3 and B4GALT4 genes in breast cancer**" under my supervision from October 2023 to March 2024 in the Department of Molecular Oncology, Cancer Institute (WIA), Adyar-Chennai.



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DECLARATION

I hereby declare that the B.Tech Thesis entitled “ **EXPRESSION ANALYSIS OF THE GENES B-4GALT-III & B-4GALT-IV IN BREAST CANCER**” submitted by me for the degree of Bachelor of Technology (B.Tech) – Biotechnology, is the record of research work carried out by me, during the period from October 2023 to March 2024 under the guidance and supervision of **Dr. G.Gopal**, professor, Department of Molecular Oncology, Cancer Institute (WIA)

Place: Chennai

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ABSTRACT:

The GALT (Galactosyltransferase) Genes encode galactose, which is normally transferred by the Galactosyltransferase enzymes. The enzymes catalyse the transfer of galactose via linkages like b1-4, b1-3, a1-3, and a1-4. In animals, the beta4GalT Genes control this transfer. Beta4GalT1, beta4GalT2, beta4GalT3, beta4GalT4, beta4GalT5, beta4GalT6, and beta4GalT7 are the seven members of the B4GalT family. They are found on the Golgi apparatus and Endoplasmic Reticulum in eukaryotes, where they play a role in lactose biosynthesis as well as the creation of other glycoconjugates. Type II transmembrane proteins make up these galactosyltransferases, a subclass of Trans-Golgi Glycosyltransferases (Glyco-T). They are distinguished by a catalytic domain, a single transmembrane domain of 20 amino acids, and a brief cytosolic section. When Real Time PCR was performed to study the gene expression of b1-4 GALT 3 and b1-4 GALT 4 genes in breast cancer. It revealed that these genes, in breast cancer are found to be down regulating, i.e., they're found to be less expressive. The same pattern was observed in Breast Cancer cell lines such as ZR75-1 and MCF7. There by, this study indicates that the b1-4 GALT 3 and b1-4 GALT 4 acts as tumor suppressors in breast cancer.

TABLE OF CONTENTS:

S.NO	CONTENTS	PAGE NO.
	ABSTRACT	i
	TABLE OF CONTENTS	ii
	LIST OF ABBREVIATIONS	vii
	LIST OF FIGURES	ix
	LIST OF TABLES	xi
1	INTRODUCTION	1-10
1.1	Breast Cancer	1
1.1.1	Where does Breast cancer start?	1
1.1.2	Metastasis of Breast cancer	2
1.1.3	Types of Breast cancer	3
1.1.3.1	In Situ Breast Cancer	3
1.1.3.2	Invasive Carcinoma	3
1.1.3.3	Invasive (Infiltrating) Ductal Carcinoma	4
1.1.3.4	Invasive Lobular Carcinoma (ILC)	4
1.2	Galactosyl Transferase	4
1.3	GALT Family of Proteins	6
1.3.1	B1-4 GAL-TS	7
1.3.1.1	B1-4 GAL-T 3	7
1.3.1.2	B1-4 GAL-T 4	8
1.4	Role of B4GALT Family of Proteins in Cancer Metabolism	9
1.5	Pathological Role of B4GALT3 and B4GALT4	10

2	LITERATURE SURVEY	11-17
2.1	Breast Cancer: A Survey	11
2.2	Diagnostic Methods	12
2.2.1	Mammography	12
2.2.2	Magnetic Resonance Imaging	13
2.2.3	Diffusion Weighted Imaging	14
2.2.4	Positron emission tomography (pet) scanning and pet in conjunction with computer aided tomography (ct) scanning (pet-ct)	14
2.3	Methodology for Cancer Classification	15
2.3.1	Filter Approach	15
2.4	Interaction between B4GALT3 and Polyacetyllactosamine	16
2.4.1	Structure of Poly -n- acetyllactosamine	17
3	AIM AND SCOPE	19
3.1	Aim	19
3.2	Objectives	19
4	MATERIALS AND METHODS	20-24
4.1	Cell Culture and Media	20
4.2	Breast Tissue Sample Preparation	20
4.3	Total RNA Isolation	21
4.3.1	RNA Isolation from Tissues	21
4.3.2	RNA Isolation from Cells	22
4.4	Agarose Gel Electrophoresis	22
4.4.1	Casting an Agarose Gel	22

4.4.2	Gel Electrophoresis run	22
4.4.3	Gel Documentation	23
4.5	cDNA Conversion	23
4.6	Designing of Real-Time quantitative Polymerase Chain Reaction (qRT-PCR) primers	23
4.7	Quantitative real-time -polymerase chain reaction (qRT-PCR)	24
5	RESULT AND DISCUSSION	25
5.1	Insilco Analysis	25
5.1.1	Insilco Analysis of B4GALT3	25
5.1.1.1	B4GALT3 gene is differently expressed in different cell lines.	25
5.1.1.2	B4GALT3 gene is differently expressed in cancer	26
5.1.1.3	B4galt3 proteomic is differentially protein expressed in cancer	26
5.1.1.4	Validation of b4galt3 gene expression in breast cancer by boxplot analysis of GEPIA	
5.1.1.5	B4GALT3 survival analysis in breast cancer	
5.1.2	Insilco Analysis of B4GALT4	29
5.1.2.1	B4GALT4 gene is differently expressed in different cell lines	29
5.1.2.2	B4GALT4 gene is differently expressed in cancer	29
5.1.2.3	B4GALT4 proteomic is differentially protein expressed in cancer	30

5.1.2.4	Validation of b4galt4 gene expression in breast cancer by boxplot analysis of GEPIA	31
5.1.2.5	B4galt4 survival analysis in breast cancer	
5.2	RNA Quantification	33
5.3	Expression Analysis of B4GALT3	34
5.3.1	Expression of B4GALT3 in Cell Lines	34
5.3.1.1	In ZR75-1	35
5.3.1.2	In MCF-7	35
5.3.2	Expression of B4GALT3 in cancerous tissue along with its corresponding paired normal	37
5.3.2.1	Highly Expressed in Tumors	42
5.3.2.2	Expression in paired normal compared to corresponding tumors	43
5.3.2.3	Low or no expression of B4GALT3 in both cancerous tissues and corresponding paired normal	43
5.4	Expression analysis of B4GALT4	44
5.4.1	Expression of B4GALT4 in Cell lines	44
5.4.1.1	In ZR75-1	44
5.4.1.2	In MCF-7	45
5.4.2	Expression of b4galt4 in cancerous tissue along with its corresponding paired normal	46
5.5	Statistical Analysis	53
5.5.1	B4GALT3 Gene Expression	53
5.5.2	B4GALT4 Gene Expression	53
6	SUMMARY AND CONCLUSION	54

REFERENCES	55
ANNEXTURE	61

LIST OF ABBREVIATIONS

B4GALT3	β - 4 Galactosyltransferase 3
B4GALT4	β - 4 Galactosyltransferase 4
BLAST	Basic local alignment search tool
BLCA	Bladder urothelial carcinoma
BRCA	Breast cancer gene
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL	Cholangio carcinoma
CPTAC	Clinical Proteomic Tumor Analysis Consortium
COAD	Colon adenocarcinoma
CRC	Colorectal cancer
DCIS	Ductal carcinoma in situ
DMEM	Dulbecco's modified eagle medium
EDTA	Ethylene diamine tetraacetate
EMSA	Electrophoretic mobility shift assay
ESCA	Esophagal carcinoma
FBS	Fetal bovine serum
GALE	Galactose epimerase
GALK	Galactokinase
GALM	Galactose mutarotase
GALT	Galactosyl transferase
GAPDH	Glyceraldehyde-3-phospate
GEO	Gene expression omnibus
GEPIA	Gene expression profiling interactive analysis
GlcNAc	N – Acetyllactosamine
GLY	D-Glucose
HER2	Human epidermal growth factor receptor

HNSC	Head and neck squamous cell carcinoma
KICH	Kidney chromophobe
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
LIHC	Liver hepatocellular carcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
ManNAc	N-Acetylmannosamine
PAAD	Pancreatic adenocarcinoma
PBS	Phosphate buffered saline
PCPG	Pheochromocytoma and paraganglioma
PRAD	Prostate adenocarcinoma
READ	Rectum Adenocarcinoma
RPMI-1640	Roswell Park Memorial Institute - 1640
SARC	Sarcomatoid carcinoma
SKCM	Human skin cutaneous melanoma
STAD	Stomach adenocarcinoma
TBE	Tris/Borate/EDTA
TCGA	The cancer genome atlas program
THCA	Thyroid carcinoma
THYM	Thymic carcinoma
UALCAN	University of Alabama and birmingham cancer data analysis portal
UCEC	Uterine corpus endometrial carcinoma
UMP	Uridine monophosphate
XYL	D-Xylose

LIST OF FIGURES:

Figure no.	Figure name	Page no.
1.1	Breast Tissue	2
1.2	Galactose Metabolism	5
1.3	Expression of B4GALT7 across pan-cancers.	10
5.1	Expression of B4GALT3 in each cell lines	25
5.2	Expression of B4GALT3 in TCGA cancer.	26
5.3	Expression of B4GALT3 in CPTAC cancer.	27
5.4	Boxplot Analysis of B4GALT3 gene in Breast Cancer.	28
5.5	Overall survival rate of patients based on the expression of B4GALT3 and prognostic markers expression.	28
5.6	Expression of B4GALT4 in each cell lines	29
5.7	Expression of B4GALT4 in TCGA cancer.	30
5.8	Expression of B4GALT4 in CPTAC cancer.	31
5.9	Boxplot Analysis of B4GALT4 gene in Breast Cancer.	31

5.10	Overall survival rate of patients based on the expression of B4GALT4 and prognostic markers expression.	32
5.11	1% Agarose Gel Electrophoresis of total RNA isolated from tumor samples.	34
5.12	Graphical representation of the fold change expression of B4GALT3 gene in cell lines in logarithmic scale.	36
5.13	Graphical representation of the fold change expression of B4GALT3 gene in tumors and their corresponding paired normal.	41
5.14	High expression of B4GALT3 in tumor and low expression of B4GALT3 in paired normal	42
5.15	B4GALT3 expressed in Paired Normal compared to expression in corresponding tumor.	43
5.16	No expression of B4GALT3 in both tumors and their corresponding paired normal.	43
5.17	Graphical representation of the fold change expression of B4GALT4 gene in cell lines in logarithmic scale.	46
5.18	Graphical representation of the fold change expression of B4GALT4 gene in tumors and their corresponding paired normal.	50
5.19	2% Agarose gel electrophoresis showing qRT-PCR amplification of B4GALT3 and B4GALT4 genes run along with DNA ladder.	52
5.20	Expression of B4GALT3 and B4GALT4 in ZR75-1 cell line.	52

LIST OF TABLES:

Table No	Contents	Page No
4.1	List of cell lines used	20
4.2	List of primers used for q-RTPCR	24
5.1	RNA Quantification in tumors and their corresponding paired normal	33
5.2	Gene Expression of B4GALT3 in ZR75-1 cell line	35
5.3	Gene Expression of B4GALT3 in MCF-7 cell line	36
5.4	Gene expression analysis of B4GALT3 in Breast Cancer tumors and corresponding paired normal.	37
5.5	Gene expression analysis of B4GALT4 in ZR75-1 cell line	44
5.6	Gene expression analysis of B4GALT4 in MCF-7 cell line	45
5.7	Gene expression analysis of B4GALT4 in Breast Cancer tumors and corresponding paired normal.	47

CHAPTER 1

INTRODUCTION

1.1 BREAST CANCER:

A Breast cancer is a type of cancer that begins in the breast, where it can start in one or both the breasts. This type of cancer is commonly diagnosed more in females when compared to males. Most of the lumps or cysts found in the breasts are found to be benign and not malignant.(McDonald et al., 2016a) Non-cancerous breast tumors refer to abnormal growths in the breast that do not metastasize beyond the breast tissue. While they are not typically life-threatening, certain types of benign breast lumps can elevate a woman's susceptibility to developing breast cancer. It is crucial for any breast lump or alteration to be examined by a healthcare provider to ascertain whether it is benign or malignant (cancerous) and if it may impact future cancer risk(Weigelt et al., 2010).

1.1.1 Where does breast cancer start?

Breast cancer originates in the cells of breast tissue, which consists of glandular tissue, connective tissue, and fat. Abnormal growth of cells can form tumors, starting in either the ducts (ductal carcinoma) or lobules (lobular carcinoma) of the breast. These cancerous cells can spread through the lymphatic system or bloodstream, leading to metastasis. Various factors contribute to breast cancer risk, including gender, age, family history, genetic mutations, hormonal factors, and lifestyle choices. Early detection through screening methods such as mammograms is crucial for effective treatment. Treatment options include surgery, chemotherapy, radiation therapy, hormone therapy, targeted therapy, and immunotherapy, tailored to each patient's specific situation. Overall, understanding breast anatomy, cancer development mechanisms, risk factors, detection methods, and treatment options is essential for addressing breast cancer effectively and improving outcomes. (Pai et al., 2021a) (Perou et al., 2000).

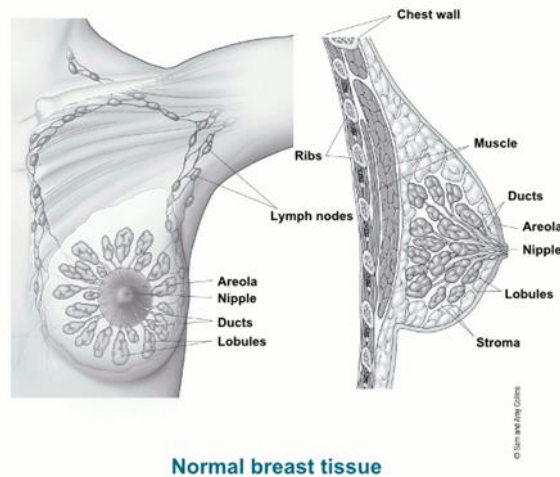


Fig 1.1 Breast Tissue

1.1.2 Metastasis of breast cancer:

Breast cancer can spread when the cancer cells get into the blood or lymph system and then are carried to other parts of the body.

The lymph (or lymphatic) system is a part of your body's immune system. It is a network of lymph nodes (small, bean-sized glands), ducts or vessels, and organs that work together to collect and carry clear lymph fluid through the body (Wiener et al., 2023a) tissues to the blood. The clear lymph fluid inside the lymph vessels contains tissue by-products and waste material, as well as immune system cells (Pai et al., 2021b).

The lymph vessels carry lymph fluid away from the breast. In the case of breast cancer, cancer cells can enter those lymph vessels and start to grow in lymph nodes. Most of the lymph vessels of the breast drain into:

- 1) Lymph nodes under the arm (axillary lymph nodes)
- 2) Lymph nodes inside the chest near the breastbone (internal mammary lymph nodes)
- 3) Lymph nodes around the collar bone (supraclavicular [above the collar bone] and infraclavicular [below the collar bone] lymph nodes)

If cancer cells have spread to your lymph nodes, there is a higher chance that the cells could have travelled through the lymph system and spread (metastasized) to other parts of your body(Rakha & Green, 2017a). Still, not all women with cancer cells in their lymph nodes develop metastases, and some women with no cancer cells in their lymph nodes might develop metastases later(Sharma et al., 2010).

1.1.3 Types of breast cancer:

Breast cancer is a heterogenous type of cancer, thereby there are many types of breast cancer, which are classified based on clinical features, tumor markers expression and histologic type. The most common type of Breast Cancer is Ductal Breast Cancer, which comprises of 75% cases and other 15% is of lobular breast cancer(Rakha & Green, 2017b).(Wiener et al., 2023b) The type of breast cancer is determined by the specific kind of cells in the breast that are affected. Most breast cancers are carcinomas. The most common breast cancers such as ductal carcinoma in situ (DCIS) and invasive carcinoma are adenocarcinomas since the cancers start in the gland cells in the milk ducts or the lobules (milk-producing glands). Other kinds of cancers can grow in the breast, like angiosarcoma or sarcoma, but are not considered breast cancer since they start in different cells of the breast(Orrantia-Borunda et al., 2022).

1.1.3.1 In Situ Breast Cancer:

The type of breast cancer can also refer to whether the cancer has spread or not. In situ breast cancer (ductal carcinoma in situ or DCIS) is a pre-cancer that starts in a milk duct and has not grown into the rest of the breast tissue.(Shinden et al., 2023) The term invasive (or infiltrating) breast cancer is used to describe any type of breast cancer that has spread (invaded) into the surrounding breast tissue.

1 in 5 new breast cancer patients are diagnosed with this type. This type is considered as stage 0 of breast cancer, which makes it easier to find the cure.

1.1.3.2 Invasive Carcinoma:

Breast cancers that have spread into surrounding breast tissue are known as invasive breast cancers.

Most breast cancers are invasive, but there are different types of invasive breast cancer. The two most common are invasive ductal carcinoma and invasive lobular carcinoma.

1.1.3.3 Invasive (infiltrating) ductal carcinoma (IDC):

This is the most common type of breast cancer. About 8 in 10 invasive breast cancers are invasive (or infiltrating) ductal carcinomas (IDC).

IDC starts in the cells that line a milk duct in the breast. From there, the cancer breaks through the wall of the duct, and grows into the nearby breast tissues. At this point, it may be able to spread (metastasize) to other parts of the body through the lymph system and bloodstream.

1.1.3.4 Invasive lobular carcinoma (ILC):

About 1 in 10 invasive breast cancers is an invasive lobular carcinoma (ILC).

ILC starts in the breast glands that make milk (lobules). Like IDC, it can spread (metastasize) to other parts of the body. Invasive lobular carcinoma may be harder to detect on physical exam and imaging, like mammograms, than invasive ductal carcinoma.(Chim-ong et al., 2014) And compared to other kinds of invasive carcinoma, it is more likely to affect both breasts. About 1 in 5 women with ILC might have cancer in both breasts at the time they are diagnosed.(McDonald et al., 2016b)

1.2 GALACTOSE METABOLISM:

Galactose is a nutrient that provides energy and is essential for the body to synthesise a large number of macromolecules. Galactose is an essential component of complex polysaccharides, which are found in many different glycoproteins, hormones, cell membrane structures, immunological determinants, endogenous lectins, and glycoconjugates.(Shauchuk et al., 2020)(G. Liu et al., 2000) Furthermore, galactose is integrated into galactolipids, which are crucial components of the central nervous system's structure(Flynn, 2022). It is also a major glycoconjugate found in prokaryotes and eukaryotes(Hennet 2002a). The significant pathway of the galactose in humans is that the conversion of galactose into glucose(Abi-Hanna and Saavedra 1998). The liver is the main site of galactose

uptake and metabolism in human beings. Liver takes up and absorbs all the circulating galactose in the blood stream. Generally, the galactose level are less or negligible in a healthy human being, but the levels of it can increase on the basis of uptake or direct ingestion of galactose or lactose. Usually, the increase or decrease of blood glucose level indicates the presence of galactose, the increase of galactose in the body will have direct effect on the blood glucose levels, this is because the crucial metabolic pathway which involves galactose metabolism results in the production of glucose(Williams, 2003).

Galactose is usually digested by human in the form of Lactose, a major constituent in human and bovine milk. Lactase, an enzyme which is located on the microvillar membrane of the small intestine, hydrolysis lactose to from glucose and galactose. After the absorption and conversion of galactose, it is then transported to liver, where it is further converted to glucose. The enzyme required for the conversion of galactose to glucose are present in many tissues, but liver play a vital role in the epimerization of the same. (BHAGAVAN 2002)

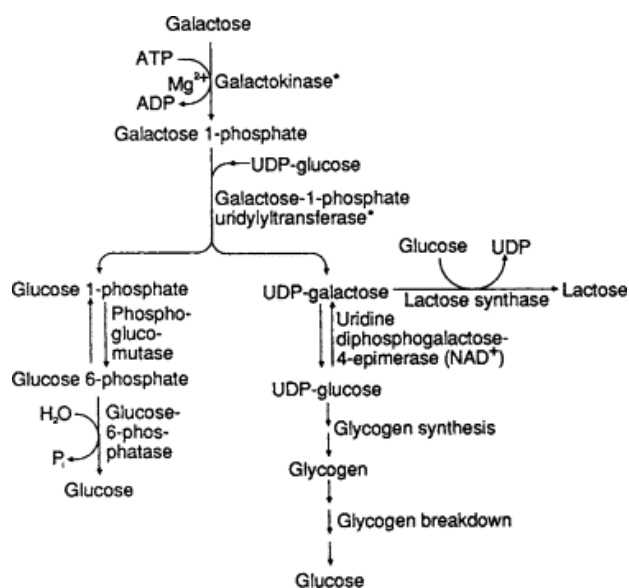
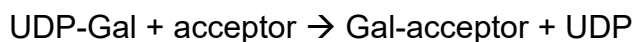


Fig 1.2 Galactose Metabolism.

1.3 GALT FAMILY OF PROTEINS:

The enzyme Galactosyltransferase also plays a role of adhesion receptor. This enzyme primarily resides in the Golgi apparatus of the cell, where it glycosylates proteins i.e., it attaches carbohydrates to the functional group of the proteins. The cytoplasmic N-terminus of this transmembrane protein gets modified by 13 amino acids by one of the two alternative initiation sites present in the mRNA for galactosyltransferase. Rather than being held in the Golgi apparatus, the longer enzyme moves to the cell membrane. The enzyme has the ability to bind oligosaccharides which terminate in N-acetylglucosamine, which is present on matrix proteins as well as the cell surface. Because there isn't a galactose-nucleotide substrate accessible outside of the cell to finish the reaction, the complex of transferase and ligand oligosaccharide in the Golgi apparatus remains stable. (Cellular Adhesion 2017).

The addition of galactose (Gal) in two anomeric configurations through α 1-2, α 1-3, α 1-4, α 1-6, or β 1-3, β 1-4 links is catalysed by several galactosyltransferase (GALT) enzymes after the standard reaction:



GALTs have a role in the production of capsular polysaccharides, pilin glycans, and the lipopolysaccharide O-antigen in prokaryotes. A GALT in the yeast *Schizosaccharomyces pombe* aids in the synthesis of galactomannan in the cell wall, and many GALTs in plants produce plastid galactolipids, galactosylated flavonols, galactomannan, and complexes such as paragaine-linked glycans. The enormous diversity of galactosylated structures is matched by many GALT gene duplication events that result in multiple groups of enzymes with various acceptor specificities and unique patterns of tissue expression, all of which are consistent with the general complexity of higher eukaryotes .(Hennet 2002b).

The activity and biological functions of galactosyltransferases have been most thoroughly characterized in mammals. In this class, Gal occurs β 1-4, β 1-3, α 1-3 and α 1-4 linked to accepting templates in various types of glycoconjugates(Hennet 2002b).

1.3.1 B1-4 GAL-TS:

Thus far, seven b1-4 GALT enzymes have been identified. The great analogy between these proteins was actually used to clone the genes encoding six members of this family. Additional related genes have not been found in recent searches of mammalian genome databases utilising known b1-4 GALT sequences as query sets. These searches do not, however, rule out the possibility that there are more b1-4 GALT genes that do not have many structural similarities with the known enzymes. Recombinant proteins have been expressed heterologously in most cases, confirming the identification of b1-4 GALT proteins. Although the enzymatic activity is clearly established by this method, it is challenging to compare the b1-4 GALT isozymes since the initial findings frequently differ in terms of the recombinant b1-4 GALT protein type and expression methods. For instance, in light of fresh research, the acceptor substrate specificity assigned to single b1-4 GALTs could need to be modified or expanded. Similarly, a recent work examining the specificity of six b1-4 GALTs produced in the same circumstances revealed that all of these enzymes had the ability to transfer Gal to N-glycan acceptors, including b4GalT6, which was previously identified as a lactosylceramide synthase (Guo et al. 2001).

1.3.1.1 β 1-4 Gal-T 3:

One of the seven genes encoding beta-1,4-galactosyltransferase (beta4GalT) is this one. They encode glycoproteins attached to type II membranes that seem to have unique selectivity for the donor substrate UDP-galactose; these glycoproteins all transfer galactose to comparable acceptor sugars, such as GlcNAc, Glc, and Xyl, via a beta1,4 linkage. Every beta4GalT has a unique role in the production of various saccharide structures and glycoconjugates (Wilkins et al., 1996). Their N-terminal hydrophobic signal region, which is characteristic of type II membrane proteins, guides the protein to the Golgi apparatus where it stays uncleaved to serve as a transmembrane anchor (*Poly-N-Acetyllactosaminy l O-Glycans Attached to Leukosialin. The Presence of Sialyl Le(x) Structures in O-Glycans - PubMed*, n.d.). The beta4GalTs are grouped into four groups based on sequence similarity: beta4GalT1 and beta4GalT2, beta4GalT3 and beta4GalT4, beta4GalT5 and beta4GalT6, and beta4GalT7. The enzyme that this gene expresses may be primarily involved in the production of poly-N-acetyllactosamine chains' first N-

acetyllactosamine unit. (Wu et al., 2020) For this gene, many variations of the alternatively spliced transcript have been discovered that encode the same protein. (B4GALT3 beta-1,4-galactosyltransferase 3 [Homo sapiens (human)] - Gene - NCBI n.d.)

Along with B4GALT2, the human B4GALT3 gene was described. A number of domains exhibit strict conservation with the other b4GalT proteins, and the total identity to the b4GalT1 protein approaches 44%. Similar to B4GALT1 and B4GALT2, the b4GalT3 gene splits into six exons and is located on human chromosome 1 band q21–q23. As an expressed recombinant soluble protein, b4GalT3 acceptors are asialo-agalacto- and GlcNAc₆-glycoproteins (Schwientek et al. 1998). Furthermore, GALT activity towards the neutral glycolipid acceptor Lc3 [GlcNAc(b1-3)Gal(b1-4)Glc(b1-)-ceramide] is significantly displayed by b4GalT3 (Liao et al., 2016). In human tissues, the b4GalT3 gene is widely expressed. As of right now, b4GalT3's biological significance is unclear (Almeida et al. 1997a).

1.3.1.2 β 1-4 Gal-T 4:

One of the seven genes encoding beta-1,4-galactosyltransferase (beta4GalT) is this one. They encode glycoproteins attached to type II membranes that seem to have unique selectivity for the donor substrate UDP-galactose; these glycoproteins all transfer galactose to comparable acceptor sugars, such as GlcNAc, Glc, and Xyl, via a beta1,4 linkage. Every beta4GalT has a unique role in the production of various saccharide structures and glycoconjugates. Their N-terminal hydrophobic signal region, which is characteristic of type II membrane proteins, guides the protein to the Golgi apparatus where it stays uncleaved to serve as a transmembrane anchor. The beta4GalTs are grouped into four groups based on sequence similarity: beta4GalT1 and beta4GalT2, beta4GalT3 and beta4GalT4, beta4GalT5 and beta4GalT6, and beta4GalT7. The enzyme that this gene expresses may be primarily involved in the production of poly-N-acetyllactosamine chains' first N-acetyllactosamine unit. For this gene, many variations of the alternatively spliced transcript have been discovered that encode the same protein. (B4GALT3 beta-1,4-galactosyltransferase 3 [Homo sapiens (human)] - Gene - NCBI n.d.)

About 40% of the protein sequences shared by b4GalT4 and the other members of the b4GalT family are identical. The human b4GalT4 gene has six exons, much like b4GalT1-3, and is located on chromosome 3 at location q13. The substantial conservation of the protein-coding sequence and the comparable genomic organisation imply that the b4GalT1-4 genes have developed through gene duplication. Every human tissue contains the b4GalT4 mRNA, albeit to varying degrees of expression. Placenta and pancreas have the highest quantities of mRNA. Along with the glycolipid acceptors Lc3 and Lc5, it demonstrated action with glycoprotein acceptors [GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (b1-)ceramide]. There is no lactose synthase activity in b4GalT4. Based on a comparative analysis, the most effective b4GalT enzyme for extending poly-N-acetyllactosamine chains on GlcNAc(b1-6) branched O-glycans was b4GalT4(Ujita et al. 1998). It is still unknown what the b4GalT4 enzyme's biological relevance is in vivo(Picardo et al. 2019).

1.4 ROLE OF GALT FAMILY OF PROTEINS IN CANCER:

GALT family proteins are observed to have different roles in different cancer types. For instance, β 1-4 galactosyltransferase - 1 is highly expressed in high metastatic lung cancer (Zhu et al. 2005). On decreasing the surface expression of β 1-4 galactosyltransferase – 1 using small interfering RNA or interfering with the surface of GALT I function by mutation inhibited cell adhesion on laminin. In case of colorectal cancer, β 1-4 galactosyltransferase – 3, it was observed that there is an up regulation of poly-N-acetyllactosamine-related glycosylation, which was associated with the metastasis of the cancer. β 1-4 galactosyltransferase – 3, an enzyme responsible for the synthesis of poly-N-acetyllactosamine plays a vital role in the progression of the metastatic stage in the colorectal cancer (Zhu et al. 2005). Apart from this, it is also responsible for the prognosis of Neuroblastoma (NB) is a neural crest-derived tumor, as poly-N-acetyllactosamine is important for the tumor progression (Chang et al. 2013) β 1-4 galactosyltransferase – 4 is a crucial promotor of Hepatocellular Carcinoma (HCC), where it knockdown downregulated the synthesis of lumican and suppressed the expressions of polo-like kinase 1 and RHAMM by regulating the transforming growth factor-beta pathway(Dai, Wang, and

Gao 2022). In colorectal cancer, apart from β 1-4 galactosyltransferase – 3, β 1-4 galactosyltransferase – 5 also plays a momentous role as a therapeutic and diagnostic biomarker(Chatterjee et al. 2019)(H. Liu et al., 2018; Sun et al., 2016; WC et al., 2015)

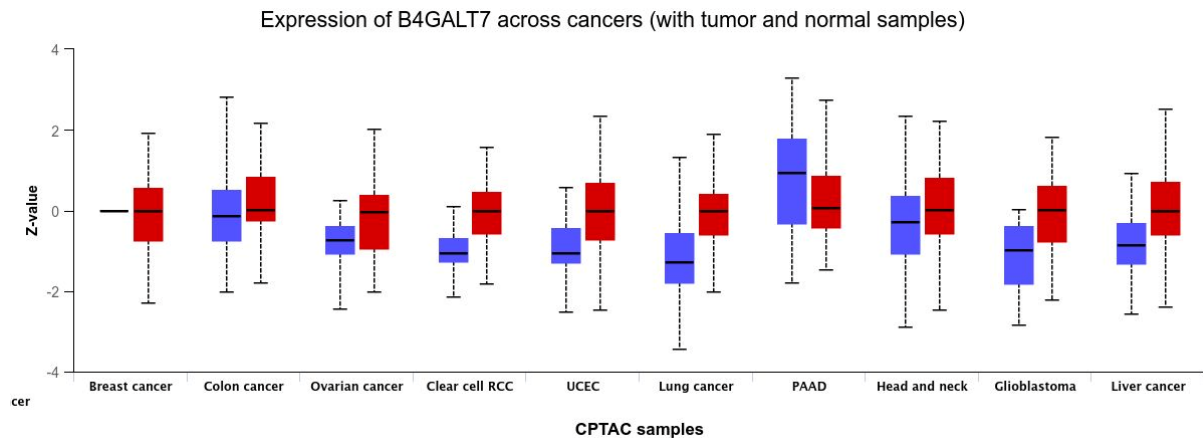


Fig1.3 Expression of B4GALT7 across pan-cancers.

1.5 PATHOLOGICAL ROLE OF B4GALT3 AND B4GALT4:

β 1-4 galactosyltransferase – 3 (B4GALT3) is crucial for the synthesis of poly-N-acetyllactosamine, which plays a vital role in the cell adhesion, signalling and recognition. On the mutation of B4GALT3, the synthesis of poly-N-acetyllactosamine reduces drastically, which further causes neurological disorders, immune dysfunction, cancer progression, glycosylation disorders. Apart from this, Sporadic inclusion body myositis, the most prevalent acquired muscle disease of ageing, is linked to GNE (UDP-GlcNAc 2-epimerase/ManNAc kinase) myopathy, a rarely occurring muscle ailments, this GNE myopathy is closely related to the mutations in GNE gene. Furthermore, it was observed that the cells having mutated GNE gene tend to synthesize distinctive poly-N-acetyllactosamine and N-linked glycan structures with more branching. The nutrient-sensing hexosamine biosynthesis pathway's essential metabolite, UDP-GlcNAc, may be impacted by GNE deprivation, however this small effect did not completely explain the shift in N-linked glycan structure(Pham et al. 2017).

CHAPTER 2

LITERATURE SURVEY

2.1 BREAST CANCER: A SURVEY:

One of the most prevalent diseases in the world today is breast cancer. In 2020, 685 000 people died worldwide from breast cancer, accounting for 2.3 million new diagnoses. Breast cancer is the most common cancer worldwide, with 7.8 million women alive as of the end of 2020 who had received a diagnosis during the previous five years. All across the world, breast cancer affects women at any age after adolescence, however its prevalence rises with age.

From the 1930s through the 1970s, when surgery alone was the main form of therapy, there was minimal improvement in the death rate from breast cancer (radical mastectomy). Survival rates started to rise in the 1990s as nations implemented early detection programmes for breast cancer that were connected to holistic treatment plans that included efficient pharmaceutical medicines. While breast cancer incidence is still high in the US and Europe, both regions have seen a decline in breast cancer death as well as incidence, various patterns can be partly attributed to variations in the rates at which various nations receive screening mammograms.

While screening mammography lowers the death rate from breast cancer based upon age, the number of mammograms performed is declining with time. Despite a number of significant side effects, including overdiagnosis, high cost, radiation exposure, and false positive biopsy suggestion, organised screening has decreased the death rate from breast cancer.

Numerous novel biomarkers, including carcinoembryonic antigen (CEA), tumour suppressor oncogene protein, human epidermal growth factor receptor 2 (HER2), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF) have been reported to be effective in identifying breast cancer in saliva. Transcriptomics, proteomics, and glycoproteomic are examples of modern technologies that can

measure hundreds of chemicals and patterns concurrently to distinguish breast cancer patients from healthy individuals.

The technique known as "metabolomics" allows for the profiling of metabolites and may be used for breast cancer screening. Because it is not possible to measure all metabolites using a single technique, profiles are created for a small number of compounds with comparable chemical characteristics, such as lipids. Sample analysis involves the application of a number of analytical techniques, such as mass spectrometry (MS) and nuclear magnetic resonance imaging. Depending on the molecules of interest, separation methods like liquid chromatography (LC) or capillary electrophoresis (CE) are employed prior to MS.

For MS-based proteomics, there are two primary methodologies. In a process known as "bottom-up proteomics," protein mixtures are broken down and the resulting peptide mixtures are analysed using either electrophoresis to separate the proteins or liquid chromatography (LC)-MS and LC-tandem mass spectrometry (LCMS/MS or shotgun approach). Individual proteins are then broken down and analysed using Matrix Assisted Laser Desorption/Ionization (MALDI)-MS, a technique known as "peptide mass fingerprinting." Proteins that are intact or entire, or a combination of proteins, are subjected to molecular mass analysis in MS mode and further fragmentation to produce partial fragments in MS/MS mode in top-down proteomics.

2.2 DIAGNOSTIC METHODS:

2.2.1 Mammography:

Mammography is a breast x-ray examination that can identify abnormalities that are benign or cancerous. To create an x-ray picture, the chest post is compressed between two plates and a little quantity of radiation is applied. Mammograms are useful for both diagnosis and screening. Mammography screening lowers mortality through early diagnosis by identifying early indicators of breast cancer before symptoms manifest. When a woman has signs of breast cancer, including a palpable lump in her breast, diagnostic mammography can assist find the disease(Jang et al., 2019).

The U.S. Preventive Services Task Force (USPSTF) released updated mammography screening guidelines in 2009, stating that women under 50 should not undergo routine mammography. This recommendation aligned with the American Cancer Society's guidelines, which suggested obtaining approximately two mammograms annually for any woman over 40 (Peisl et al., 2019). Furthermore, because the radiologist interprets the results subjectively, breast density cannot be utilised to deduce the information from her mammography.

For instance, despite having the same breast density evaluation, patients may undergo rather distinct mammograms and receive quite varied findings from each one. Prior research produced statistics on glandular tissue volume based on mammography results. These automated techniques for determining breast density, however, are insufficient to forecast the occurrence of breast cancer. It has recently been demonstrated that mammography picture contrast may be greatly increased by using gold-based nanoformulations (Giampietro et al., 2020).

2.2.2 Magnetic resonance imaging:

Breast MRI is a non-invasive, non-ionizing imaging technique that creates precise pictures of the breast's internal components using magnetic fields and low-energy radiofrequency waves. MRIs can be used to detect metastatic tumours and gauge the extent of breast cancer in women who have already received a diagnosis. MRI was used to precisely identify and measure tumours less than 2 cm. (Gao et al., 2021)

However, because of aberrant breast tissue around the lesion, bigger breast tumours are frequently exaggerated, which might result in a greater likelihood of mastectomy. The American College of Radiology suggests using breast MRI for a number of purposes, one of which being high-risk population screening. Suspected breast cancers that frequently escape clinical evaluation, mammography, and ultrasound can be found with MRI. Contrast compounds based on Fe₃O₄, gadolinium (III), and Mn (II) are frequently utilised for preoperative assessment, particularly when imaging the breast's axillary lymph nodes. (Muradali et al., 2023)

To reduce the possibility of off-target toxic effects and increase specificity for breast cancer, these contrast agents can be encapsulated in breast cancer-targeting

polymeric carriers. Breast MRI is often used to diagnose breast cancer due to its high sensitivity and low specificity, leading to an increase in incidental findings. It is essential to evaluate these findings histologically before surgical treatment.

2.2.3 Diffusion weighted imaging:

Diffusion-weighted imaging (DWI) is a non-contrast magnetic resonance imaging technique that improves upon standard breast MRI by using the diffusion of water molecules to provide contrast in magnetic resonance images. Enhancing the ability to distinguish between benign and malignant breast lesions as well as assessing and forecasting the effectiveness of treatment are possible advantages of DWI technology. Particularly in cases of thick breasts, DWI made it possible to identify breast cancer.

Nevertheless, DWI's sensitivity is often different from contrast-enhanced MRI's. The widespread use of breast DWI has been hampered by picture quality difficulties, which have been addressed in part by technological advancements. Though its full potential as a non-radioactive, accurate imaging method has not yet been realised, DWI has great promise. Currently, in-depth analyses and clinical research are needed to prove that DWI may help with accurate illness diagnosis.

2.2.4 Positron Emission Tomography (PET) scanning and PET in conjunction with Computer aided Tomography (CT) scanning (PET-CT):

It is commonly acknowledged that PET imaging is a crucial clinical tool in the field of cancer. While there are other PET radiotracers available for non-invasively examining tumour metabolism in vivo, the most commonly utilised one is 2-deoxy-2-(18F) fluoro-D-glucose (FDG).(Ikejiri et al., 2022) It is a PET radiotracer that has FDA approval and is in use. Cancer cells have an enhanced metabolism of glucose. Compared to normal cells, cancer cells proliferate rapidly and have a greater rate of glucose consumption. Because the FDG PET radiotracer enters cells through glucose transporters, tumour cells absorb more of it than do healthy cells. Anticipations are negatively correlated with FDG consumption.(Bertagna et al., 2020)

PET-CT is a nuclear medicine technique that combines PET with CT to provide incredibly precise body pictures. The investigation of primary tumours may now be

done clinically thanks to advancements in breast PET (positron emission mammography) scanner sensitivity and spatial resolution. 18F-FDG-PET/CT hybrid imaging has been demonstrated in several investigations to offer information on cellular glucose absorption, which is elevated in malignant tumours. According to Jorgensen et al., following nanoparticle-assisted photothermal therapy, tumour cells' absorption of 18F-FDG was considerably decreased, indicating that the marker may be utilised to measure treatment response. PET-CT tests are used by doctors to identify cancer, determine a course of therapy, evaluate its efficacy, and oversee continuing care.(Ulaner et al., 2021)

2.3 METHODOLOGY FOR CANCER CLASSIFICATION:

2.3.1 Filter approach:

Regardless of the classifier, it assesses features based on inherent characteristics of the data, such as consistency, correlation, and distance. Their performance is weak, but they can generalise well and need a lot of computing power. There are two types of filter approaches: multivariate and univariate. To assess the degree of correlation between a feature and the end variable, univariate feature selection looks at each feature separately. Mutual information (MI) and information gain (IG) are common forms.

- The correlation between two variables is measured by MI.

Stated differently, it quantifies the degree of knowledge that one variable (X) has about another variable (Y). The relationship between genes and categorization categories is measured by gene selection. There is more gene information the higher the MI number.

- Aspects that are highly associated to a class are those that contain information on the free site for psychological animal fortune-telling; irrelevant aspects don't supply any information.

The entropy value, which depicts the impurities in a particular sample, is used to calculate the IG value. Following that, a threshold is established, and characteristics with values higher than are chosen. Multivariate assesses characteristics in different situations. The most popular methods are:

- Maximum Relevance and Minimum Redundancy (mRMR), which favours characteristics with strong and weak correlations to the class. Choosing both highly relevant and highly correlated characteristics is illogical since, despite their strong correlation, they don't offer any new information. Instead, they make the model more complicated and raise the likelihood of overfitting.

- a free website where animals reveal psychological fortunes.
- a free website where animals reveal psychological fortunes.

The following theories are used to assess features. Features that are substantially linked with classification but uncorrelated with one another make up a good feature subset. Stated differently, characteristics that have a low correlation with the class are considered irrelevant but features that are present and have a high correlation are informative features.

- A multivariate technique based on symmetric uncertainty (SU), the fast correlation-based filter (FCBF) chooses characteristics that have a strong correlation with a class.

After that, we use heuristics to eliminate characteristics that are unnecessary and keep just those that are pertinent to the class.

2.4 INTERACTION BETWEEN B4GALT3 AND POLY-ACETYLLACTOSAMINE:

The B4GALT3 gene in human codes for the enzyme B-4Galactosyltransferase III. This gene, one of the seven in the B4GALT family, encodes type II membrane-bound glycoproteins that seem to have unique selectivity for the donor substrate UDP-galactose; these acceptor sugars, which include GlcNAc, Glc, and Xyl, all transfer galactose via a beta1,4 linkage. This gene primarily codes an enzyme that might be mainly involved in the synthesis of the first N-acetyllactosamine unit of poly-N-acetyllactosamine chains(B4GALT3 beta-1,4-galactosyltransferase 3 [Homo sapiens (human)] - Gene - NCBI n.d.).

Keratan Sulfate, a glycoprotein(Leppänen et al., 2002), which consists of the repeating units of N-Acetyllactosamine is synthesized from four enzymes, where two glycosyltransferases (β -1,3-N-acetylglucosaminyltransferase and β -1,4-

galactosyltransferase), which transfer GlcNAc and Gal to a nonreducing terminus of a carbohydrate core structure connected to carrier proteins, are involved in elongation of the poly-N-acetyllactosamine backbone of KS (Linhardt and Weïwer 2007a). Two sulfotransferases (GlcNAc 6-O-sulfotransferase and Gal 6-O-sulfotransferase), which transfer a sulfo group to the 6-O-position of GlcNAc or Gal, are involved in the modification of the resulting polysaccharide and allow the formation of KS which is composed of both mono- and disulfated disaccharides. (Linhardt and Weïwer 2007b).

Found in cartilage, cornea, and brain, keratan sulphate is a β -1,3-linked poly-N-acetyllactosamine with sulphate residues on the 6-positions of both galactose and N-acetylglucosamine. (Maemura & Fukuda, 1992) Light may flow through the collagen fibrils in the eye because keratan sulphate proteoglycans keep them spaced regularly. Defects in sulfation lead to distortions in the organisation of the fibrils and corneal opacity, which can lead to macular corneal dystrophy (Stick and Williams 2009).

2.4.1 Structure of Poly-n-acetyllactosamine synthesis

N-acetyllactosamine repetitions combine to form poly-N-acetyllactosamine, a unique carbohydrate that serves as the foundation for further modifications like sialyl Lex. Core 2 branched oligosaccharides, which are produced by core 2 β -1,6-N-acetylglucosaminyltransferase, have the potential to create poly-N-acetyllactosamines in mucin-type O-glycans.

Starting with a core 2 branched oligosaccharide, $\text{GlcNAc}\beta 1\rightarrow 6(\text{Gal}\beta 1\rightarrow 3)\text{GalNAc}\alpha\rightarrow \text{R}$ (Sasaki et al. 1997), it was observed that the production of poly-N-acetyllactosamine was exceedingly inefficient using a β -1,4-galactosyltransferase ($\beta 4\text{Gal-TI}$) found in milk and the recently cloned β -1,3-N-acetylglucosaminyltransferase. We tested novel members of the $\beta 4\text{Gal-T}$ gene family for this synthesis since galactosylation was deemed inefficient because most synthesised oligosaccharides had N-acetylglucosamine at the nonreducing ends.

It was discovered that $\beta 4\text{Gal-TIV}$ was the most effective recombinant $\beta 4\text{Gal-Ts}$ and synthetic acceptor for adding a single galactose residue to $\text{GlcNAc}\beta 1\rightarrow 6(\text{Gal}\beta 1\rightarrow 3)\text{GalNAc}\alpha\rightarrow \text{R}$. Furthermore, in core 2 branched

oligosaccharides, β 4Gal-TIV (Almeida et al. 1997b) and β -1,3-N-acetylglucosaminyltransferase were able to synthesise poly-N-acetyllactosamine (Togayachi et al. 2018).

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM

To analyze the expression levels of GALT gene and do a comparative study between breast tumor and pair normal cells.

3.2 OBJECTIVES:

- To study the expression status of B4GALT 3 and 4 genes in breast cancer cell lines.
- To compare the gene expression of B4GALT 3 and 4 genes in breast cancer tissues using Real time PCR.
- To perform Insilco analysis of B4GALT 3 and 4.
- To study the gene expression and survival status in other cancer subtypes.

CHAPTER 4

MATERIALS AND METHODS

4.1 CELL CULTURE AND REAGENTS:

Human derived cell lines (Table 4.1) were maintained in T-25 cm² flasks (Corning Inc., USA) with either Roswell Park Memorial Institute -1640 (RPM1-1640) (HI Media, India) or Dulbecco's Modified Eagle Medium (DMEM) (HI Media, India), supplemented with 10% Fetal bovine serum (FBS) (GIBCO, USA) containing 100 mg/L streptomycin and 100 mg/L penicillin (HI Media, India). The cultures were kept at 37°C and 5% CO₂ in an incubator with humidification. 80 to 90% confluent cells were prepared for sub-culturing by first washing them in sterile phosphate-buffered saline (PBS) and then adding 0.5 to 2 mL of Trypsin-EDTA solution [0.25% w/v Trypsin (Sigma Aldrich, India) and 0.001% w/v ethylene diamine tetraacetate (EDTA) (MB Biomedicals, India) dissolved in PBS and sterilised with a 0.2 µm filter]. To inactivate trypsin, the cells were centrifuged at 800 rpm for two minutes, and the pellet was then suspended in a growth medium containing 10% FBS. Liquid nitrogen was used to freeze cell line stocks, which were prepared as follows: 70% FBS, 20% DMEM, and 10% dimethyl sulfoxide.

Table 4.1 List of Cell lines used.

Types of Cell Lines	Cell Lines	Medium Used
Control	HEK293	DMEM with 10% FBS
Breast Cancer Cell Lines	MCF7 and ZR75-1	DMEM with 10% FBS

4.2 BREAST TISSUE SAMPLE PREPARATION:

Tissue samples were acquired after obtaining the consent from the informed breast cancer patients who underwent Modified Radical Mastectomy without any previous treatment. The tissues which were adjacent to the tumor were collected and assessed to be histologically normal cells and were termed and used as paired-normal (PN) samples. In total, 20/31 patients were diagnosed with Invasive Ductal

Carcinoma, 7/31 patients were diagnosed with Invasive Breast Carcinoma, 2/31 patients were diagnosed with Ductal Carcinoma In Situ (DCIS) , 2/31 patients were diagnosed with Mucinous Carcinoma, one patient was diagnosed with Metaplastic (spindle cell) Carcinoma. The tissues obtained were stored in RNA later for 24 hours in order to preserve the RNA of the tissues. Then, the RNA later was removed from the samples and were frozen in -80°C.

4.3 TOTAL RNA ISOLATION:

4.3.1 RNA isolation from tissues:

The total RNA was isolated from the tissues using Trizol (Sigma-Aldrich). The tissues were first frozen using liquid N₂ . The tissues were then grinded into fine granules using mortar and pestle. These granules were then suspended in Trizol (Sigma-Aldrich) for 15-20 minutes. They were then centrifuged for 3 min at 10,000 rpm at 4°C. The supernatant was collected in fresh centrifuge tubes and the pellets were discarded. 1/10th volume of total volume of 3-Bromo-4-Chloro Propane was added to the tissue suspended in the Trizol and was mixed gently and incubated for 5 minutes in room temperature. Then, the solution was centrifuged at 10,000 rpm for 15 minutes at 4°C. The aqueous phase was pipetted out without disturbing the other layers and transferred to a fresh 1.5 mL tubes and placed on ice. An equal volume of the aqueous phase of Isopropanol was added to the aqueous phase. After mixing it gently, they are left for incubation for 10 minutes. Then, the samples are centrifuged at 10,000 rpm for 20 minutes at 4°C. The white pellet obtained was washed twice using 75% ethanol. Then the pellet was air-dried and dissolved in 15 µL of sterile nuclease-free waster. The RNA concentration was quantified using Nanodrop ND 2000 spectrophotometer (Thermo Scientific, USA). Sterile nuclease-free water was used to set blank and 1 µL of the sample was used to estimate the concentration of RNA in ng/µL. Absorbance at 260 nm/280 nm ratio between 1.8 and 2 indicated the purity of the RNA isolated from the samples.

4.3.2 RNA isolation from cells:

Total RNA was isolated using Trizol reagent (Sigma-Aldrich). Cells suspended in Trizol were added to a one-tenth of 3-Bromo-4-Chloro Propane, mixed gently, and centrifuged at 10,000 rpm for 10 min at 4°C. The aqueous phase was pipetted out without disturbing the contents and transferred to a fresh 1.5 mL tube and placed on ice. Then, isopropanol was added to the aqueous phase, after gentle a vortex, it was left for 10 minutes for incubation, the cell lines were then centrifuged at 10,000 rpm for 15-20 min at 4°C. The white pellet thus formed was washed twice with 75% ethanol. Then the pellet was air-dried and dissolved in 15 µL of sterile nuclease-free water. The RNA concentration was quantified using Nanodrop ND 2000 spectrophotometer (Thermo Scientific, USA). Sterile nuclease-free water was used as blank and 1 µL of the sample was used to estimate the concentration of RNA in ng/µL. Absorbance at 260 nm/280 nm ratio between 1.8 and 2 indicated the purity of the isolated RNA.

4.4 AGAROSE GEL ELECTROPHORESIS:

4.4.1 Casting an agarose gel:

Based on the number of RNA, that needed to be assessed for its integrity, the number of wells in the agarose gel is decided. For 13 wells, 2% of the agarose gel is prepared by carefully weighing 2.6 gm of agarose (Cytvia, USA) to 130mL of 1x Tris/Borate/EDTA (TBE) buffer (pH 7.6) that was made out of 0.13 M Tris, 5 mM boric acid, and 2.5 mM EDTA, whereas for 6 wells, 1% of the gel is prepared by carefully weighing 0.7 gm of agarose to 70ml of of 1x Tris/Borate/EDTA (TBE) buffer (pH 7.6), the solution was then microwaved for 1 minute 30 seconds, until the agarose was completely dissolved. Depending on the quantity of RNA, for 5 µl, 3 µl of firefly dye (Biokart,India) was added.

4.4.2 Gel electrophoresis run:

The gel boat was then fixed inside the Gel tank that contains 1X TBE buffer and the comb was removed. Initially, 4 µl of 10 kbp Ladder (Takara, Japan) along 2 µl firefly was loaded to compare the base pair length of samples, then 5 µl of the RNA sample

along with 2 µl of firefly dye were loaded into consecutive wells. The leads were connected, and the gel was run at a constant voltage of 100 Volts for 30 minutes.

4.4.3 Gel documentation:

The gel was visualized and documented using the ChemiDoc™ MP imaging system (Biorad, USA). under UV absorbance.

4.5 cDNA CONVERSION:

1 µg of total RNA underwent cDNA conversion using the PrimeScript™ RT-PCR Kit (DSS Takara Clonetechn, Japan) following the manufacturer's instructions in a two-step process. The reaction mixture consisted of 1 µg of total RNA, 1 µl of oligoDT primer (2.5 micromolar), and 1 µl of dNTP mixture (10 millimolar), with the volume made up to 10 µl using sterile nuclease-free water. This mixture underwent denaturation and annealing at 65 °C for 5 minutes followed by 4 °C for 10 minutes in a thermal cycler. Subsequently, the reaction mixture was combined with 4 µl of 5X PrimeScript buffer, 0.5 µl of RNase inhibitor (40 U/microliter), 0.5 µl of Reverse Transcriptase, and made up to a final volume of 20 µl with sterile nuclease-free water.

4.6 DESIGNING OF REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION (QRT-PCR) PRIMERS:

The primers for the two B4GALT genes and housekeeping genes are given in Table 2.2, which were designed using the Integrated DNA Technologies (Coralville, Iowa, USA) primer designing tool. The primers were validated by primer BLAST (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>) and then given for synthesizing to Eurogentec, Belgium. The primers were dissolved in sterile nuclease-free water and diluted to a working concentration of 10 µM for further use.

Table 4.2 – Primers for qRT-PCR

Gene	Primers for qRT-PCR
B4GALT3	FP:5'TCCTCAAGGTCTGCCCTACTGT3' RP:5'ATTCCGCTCCACAATCTCTGCC3'
B4GALT4	FP:5'CTCTGACTAATGAAGCATCCACG3' RP:5'CTGCCTGTACCTCTTCCAAAGTG3'
GAPDH	FP: 5'GTCTCCTCTGACTTCAACAGCG3' RP: 5'ACCACCCTGTTGCTGTAGCCAA3'

4.7 QUANTITATIVE REAL-TIME -POLYMERASE CHAIN REACTION (QRT-PCR):

qRT-PCR was carried out with the gene-specific primers for the two B4GALT genes (B4GALT3 and B4GALT4) and GAPDH as the normalising control genes, by utilizing the Promega GoTaq® qPCR Master Mix (Madison, Wisconsin, US) with respect to manufacturer's protocol in duplicates and triplicates. 10 µL of master mix, 1 µL of forward primer (10 µM), 1 µL of reverse primer (10 µM), 1 µL of cDNA template (50 ng) was prepared and was made up to 20 µL with sterile-nuclease free water. PCR amplification was conducted using the ABI Prism 7000 Sequence Detection System from Applied Biosystems, based in the USA. The thermal-cycling conditions includes 95oC for 2 min (polymerase activation), 95oC for 15 seconds, 60oC for 1 minute for 40 cycles.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 INSILCO ANALYSIS

5.1.1 *Insilico analysis of B4GALT3:*

5.1.1.1 *B4GALT3 gene is differently expressed in different cell lines:*

The B4GALT3 gene expression in cell lines was analysed using GOBO BRCA datasets. It showed that in HCC70, HCC1 569, HCC2 157, HCC1 187, SUM190PT, MCF12A, MCF10A, ZR751, SUMA4PE, HCC1428, HCC202, CAMA1, MADAMB4533, T47D, BT483, AU565, SKBR3, MDAMB175VII, ZR45B, LY2, X60MPE, BT474, UACC812, SUM185PE and ZR7530, B4GALT3 gene was highly expressed. Whereas in cell lines like, MDAMB468, BT20, HCC1937, SUM225, HCC1954, HCC1143, MDAMB157, MDAMB436, BT549, HBL100, SUM1315MO2, MDAMB435, SUM149PT, HCC1500, SUM159PT, MDAMB415, MDAMB134VI, MDAMB361, SUM52PE, MCF7, HCC1007, HCC21, B4GALT3 gene showed an downregulation.

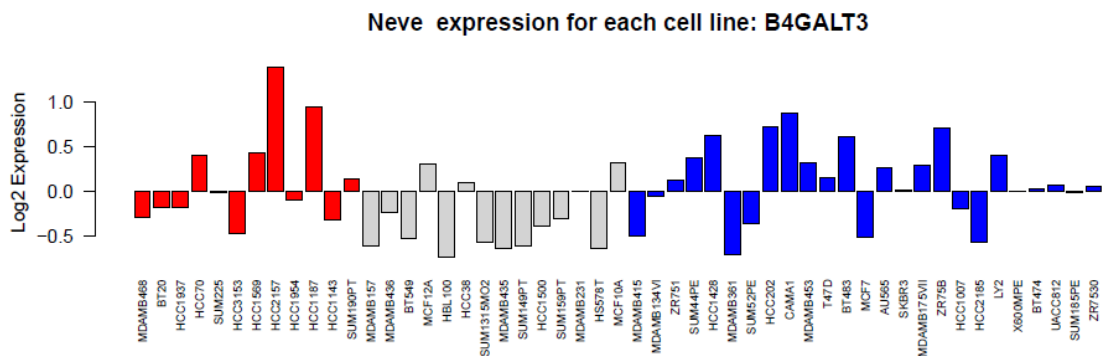


Fig 5.1 Expression of B4GALT3 in each cell lines

(credits: <https://co.bmc.lu.se/gobo/>)

5.1.1.2 B4GALT3 gene is differently expressed in cancer:

The B4GALT3 gene expression was analysed using UALCAN TCGA datasets. It can be observed that in Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), Uterine Corpus Endometrial Carcinoma (UCEC), Cholangio carcinoma (CHOL), in Glioblastoma multiforme (GBM), Pheochromocytoma and Paraganglioma (PCPG), in Colon adenocarcinoma (COAD), Kidney Chromophobe (KICH), Kidney renal papillary cell carcinoma (KIRP), Lung squamous cell carcinoma (LUSC), Breast invasive carcinoma (BRCA), Prostate adenocarcinoma (PRAD), Kidney renal clear cell carcinoma (KIRC), B4GALT3 was upregulated significantly. [Table 5.] [Fig 5.2]. The analysis TCGA datasets have revealed that the B4GALT3 to be differentially expressed, and majority of cancers showing upregulation, elucidating its involvement in the tumour progression.

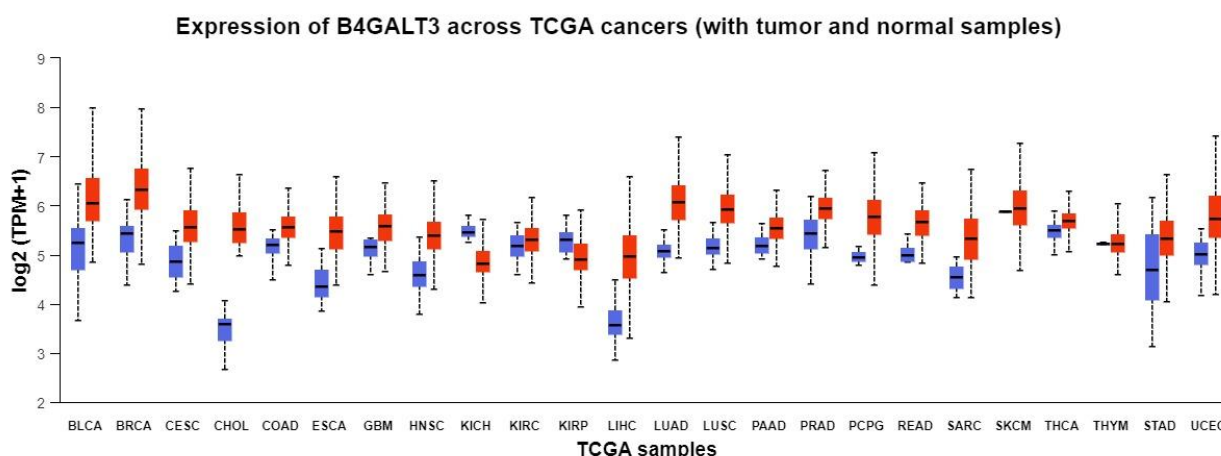


Fig 5.2 Expression of B4GALT3 in TCGA cancer.

(credits: <http://ualcan.path.uab.edu/>)

5.1.1.3 B4GALT3 proteomic is differentially protein expressed in cancer:

The B4GALT3 proteomic expression was analysed using UALCAN CPTAC datasets. It showed that in Colon Cancer, Ovarian Cancer, Uterine Corpus Endometrial Carcinoma (UCEC), Lung Cancer, Head and Neck Cancer, Glioblastoma and Liver Cancer, B4GALT3 was upregulated. Whereas in Breast

Cancer, Pancreatic adenocarcinoma (PAAD), B4GALT3 was found to be downregulating [Table 5.] [Fig 5.3]. According to the analysis of CPTAC databases, it is observed that the expression of B4GALT3 was found to be variable in accordance with different types of cancers, where in majority types of cancers, it is found to upregulating, implying The differential expression of B4GALT3 indicates its involvement in the tumor progression and can be potential as a target for therapeutic intervention.

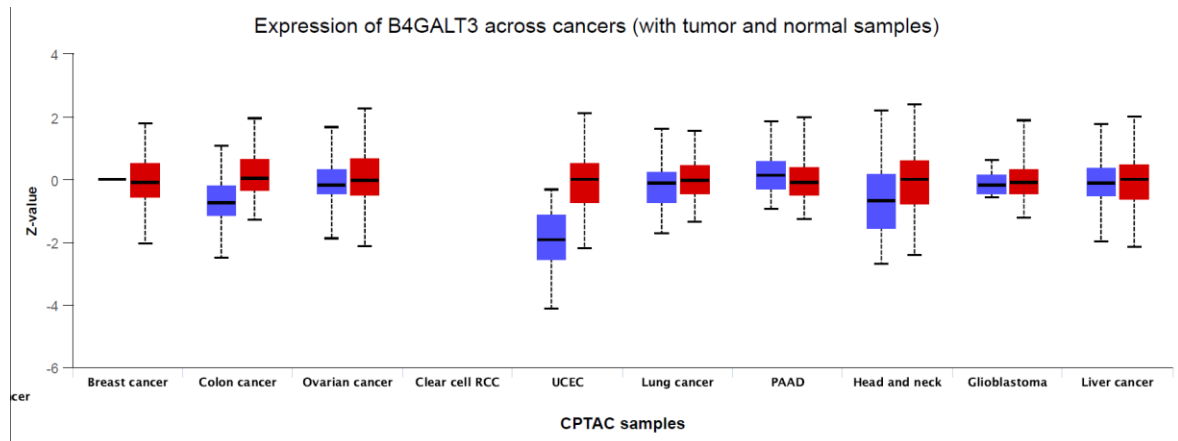


Fig 5.3 Expression of B4GALT3 in CPTAC cancer.

(credits: <http://ualcan.path.uab.edu/>)

5.1.1.4 Validation of B4GALT3 gene expression in breast cancer by boxplot analysis of GEPIA:

The gene expression of B4GALT3 gene was also visualised and analysed by Boxplot analysis in GEPIA. It showed an upregulation of B4GALT3 gene, which is found to be similar to that of UALCAN TCGA analysis.

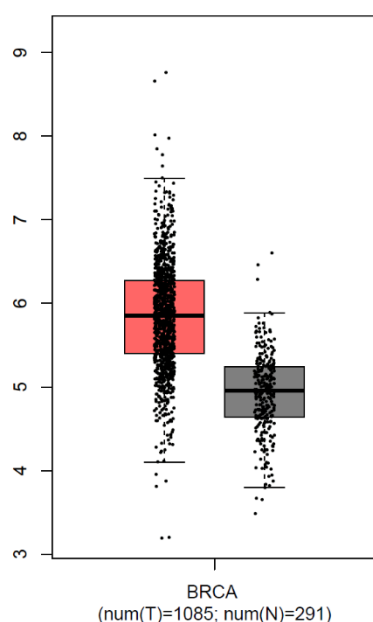


Fig 5.4 Boxplot Analysis of B4GALT3 gene in Breast Cancer.

(credits: <http://gepia.cancer-pku.cn/detail.php?gene=B4GALT3>)

5.1.1.5 B4GALT3 survival analysis in breast cancer:

Survival analysis using GEPIA in Breast Cancer showed that the markers like Hormone Receptors (HR) [Fig5.5] showed no significant value. Thereby, on conclusion, it indicated that the Hazard Ratio (HR) is low, indicating that the chances of the relapse of the breast cancer is less.

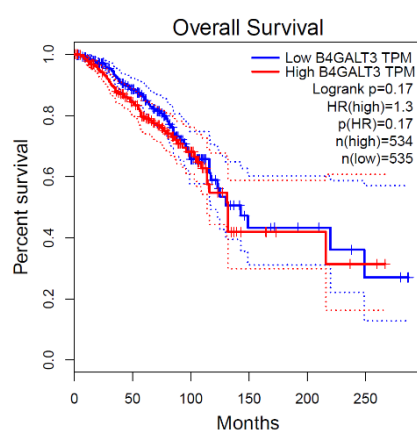


Fig 5.5 Overall survival rate of patients based on the expression of B4GALT3 and prognostic markers expression.

Pheochromocytoma and Paraganglioma (PCPG), , Kidney Chromophobe (KICH), Kidney renal papillary cell carcinoma (KIRP), Lung squamous cell carcinoma (LUSC), Breast invasive carcinoma (BRCA), , B4GALT4 was upregulated significantly. [Table 5.] [Fig 5.2]. Moreover, in Colon adenocarcinoma (COAD), Kidney renal clear cell carcinoma (KIRC), Prostate adenocarcinoma (PRAD), Thyroid Carcinoma (THCA), B4GALT4 gene was found to downregulated. The analysis TCGA datasets have revealed that the B4GALT4 to be differentially expressed, and majority of cancers showing upregulation, elucidating its involvement in the tumour progression.

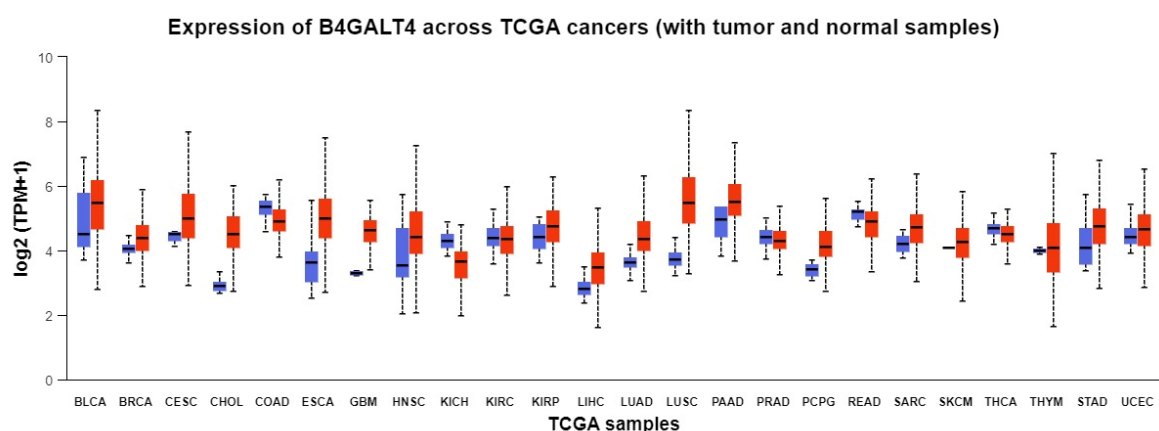


Fig 5.7 Expression of B4GALT4 in TCGA cancer.

(credits: <http://ualcan.path.uab.edu/>)

5.1.2.3 B4GALT4 proteomic is differentially protein expressed in cancer:

The B4GALT4 proteomic expression was analysed using UALCAN CPTAC datasets. It showed that in Uterine Corpus Endometrial Carcinoma (UCEC), Lung Cancer, , Pancreatic adenocarcinoma (PAAD), Glioblastoma and Liver Cancer, B4GALT4 was upregulated. Whereas in Colon Cancer, Ovarian Cancer, Head and Neck Cancer, B4GALT4 was found to be downregulating. However, in Breast Cancer, it is found to have no difference in the expression of the B4GALT4 gene in tumor when compared to its corresponding normal. [Fig 5.3]. According to the analysis of CPTAC databases, it is observed that the expression of B4GALT4 was found to be variable in accordance with different types of cancers, where in majority

types of cancers, it is found to upregulating, implying The differential expression of B4GALT4 indicates its involvement in the tumor progression and can be potential as a target for therapeutic intervention.

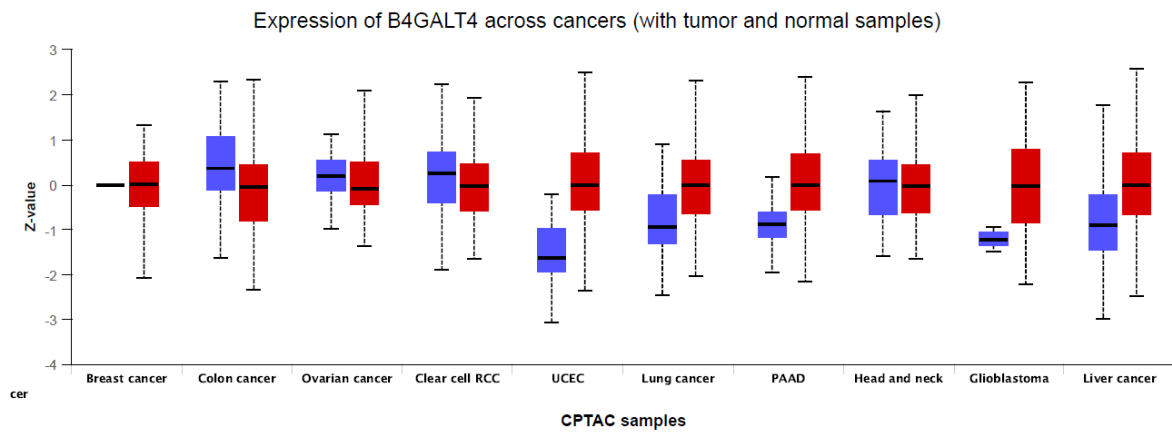


Fig 5.8 Expression of B4GALT4 in CPTAC cancer.

(credits: <http://ualcan.path.uab.edu/>)

5.1.2.4 Validation of B4GALT4 gene expression in breast cancer by boxplot analysis of GEPIA:

The gene expression of B4GALT4 gene was also visualised and analysed by Boxplot analysis in GEPIA. It showed an upregulation of B4GALT3 gene, which is found to be similar to that of UALCAN TCGA analysis.

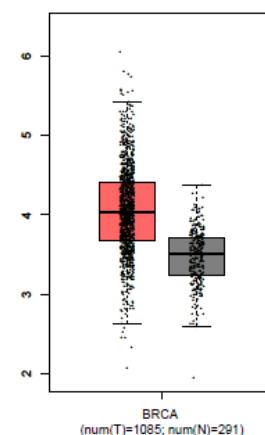


Fig 5.9 Boxplot Analysis of B4GALT4 gene in Breast Cancer.

(credits: <http://gepia.cancer-pku.cn/detail.php?gene=B4GALT4>)

5.1.2.5 B4GALT4 survival analysis in breast cancer:

Survival analysis using GEPIA in Breast Cancer showed that the markers like Hazard Ratio (HR) [Fig5.5] showed a significant value, $p = 0.024$. Thereby, on conclusion, it indicated that the Hazard Ratio (HR) was found to be significant. On conclusion, it increases the probability of relapse of the Breast Cancer (BC).

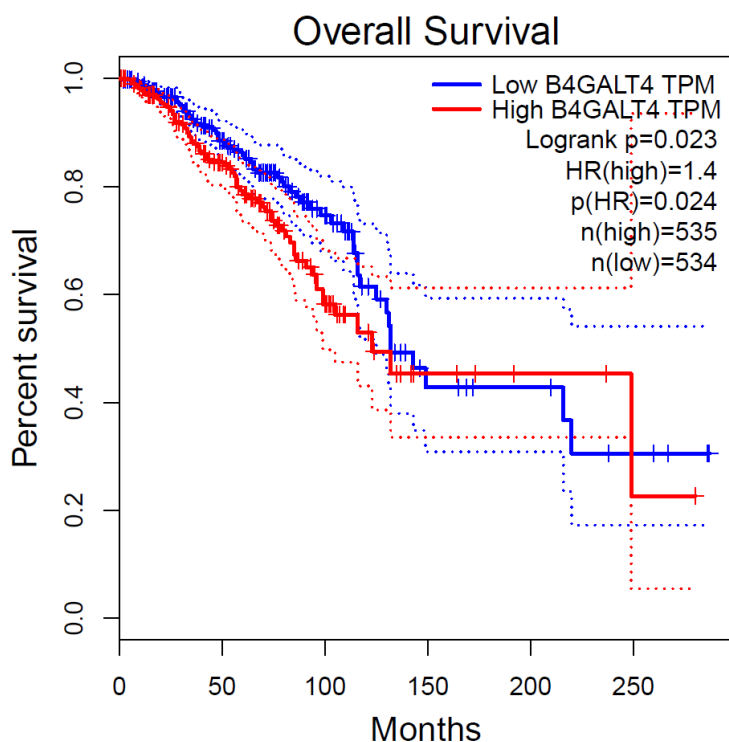


Fig 5.10 Overall survival rate of patients based on the expression of B4GALT4 and prognostic markers expression.

5.2 RNA QUANTIFICATION:

Table 5.1 RNA Quantification in tumors and their corresponding paired normal

No	RNA Quantification (T)	RNA Quantification (PN)	A260/A280 (T)	A260/A280 (PN)
1.	185.4	74.2	2.15	2.17
2.	618.6	91.6	1.56	1.97
3.	279.6	65	1.99	1.67
4.	319.2	69.9	2.09	2.05
5.	155.7	52.9	2	2.43
6.	181.7	43.3	1.7	2.38
7.	225.1	81.1	2.12	2.21
8.	391	228.2	2.02	2.33
9.	150.2	105.6	2.24	2.24
10.	2020.7	315.2	1.02	2.3
11.	214.3	198.1	2.11	2.15
12.	1210.7	214.4	2.13	2.22
13.	1166.9	270.3	1.95	2.28
14.	126.8	578.5	2.02	2.49
15.	801.6	313.1	1.97	2.23
16.	808	218.2	2.28	2.16
17.	1691.5	33.9	2.04	2.06
18.	102.5	356.2	2.04	2.45
19.	275.8	218.3	2.12	2.31
20.	1804	119.1	2	2.13
21.	2444.4	1791.7	1.51	1.93
22.	1610.4	155.8	2.1	2.45
23.	947.4	198.9	1.94	2.01
24.	551.8	102.8	2.04	2.14
25.	376.3	197.1	2.06	2.22
26.	310.3	195.2	2.16	2.2
27.	67.9	225	1.82	2.38
28.	167.6	21.5	2.15	2.05
29.	286.1	103.7	2	2.28
30.	925.6	37.6	2.05	2.15
31.	1960.7	322.1	1.16	2.15

The RNA concentration of the measured samples consistently showed higher values for the tumors and comparatively lower values in paired normal. The lower concentration of RNA can be attributed to the fact that; the human breast tissue is predominantly composed of adipose tissues. An absorbance ratio of ~ 2.0 is generally considered as pure for RNA.

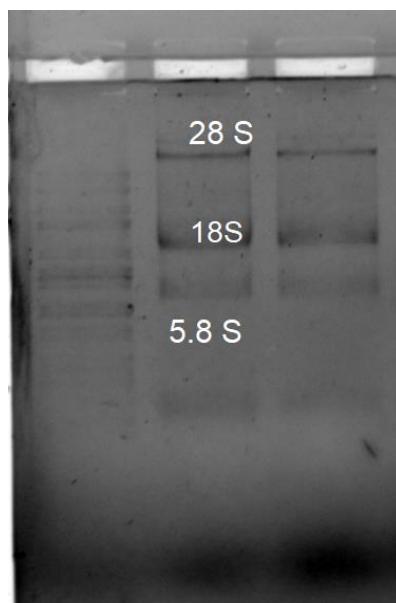


Fig 5.11 1% Agarose Gel Electrophoresis of total RNA isolated from tumor samples.

5.3 EXPRESSION ANALYSIS OF B4GALT3:

A qRT-PCR was performed with gene specific primers [table 4.2] for the gene B4GALT3, as well as GAPDH as a normalising control gene. By using the Promega GoTaq® qPCR Master Mix (Madison, Wisconsin, US) with respect to manufacturer's protocol in duplicates. The layout was planned in 96 well plate, where 10 μ L of master mix, 1 μ L of forward primer (10 μ M), 1 μ L of reverse primer (10 μ M), 1 μ L of cDNA template (50 ng) was prepared and was made up to 20 μ L with sterile-nuclease free water were added. qRT-PCR amplification was conducted using the ABI Prism 7000 Sequence Detection System from Applied Biosystems, based in the USA. The thermal-cycling conditions includes 95°C for 2 min (polymerase activation), 95°C for 15 seconds, 60°C for 1 minute for 40 cycles.

5.3.1 Expression of B4GALT3 in cell lines:

Having the cell lines – ZR75-1 and MCF7 as template, qRT-PCR was performed, and the results were interpreted.

5.3.1.1 In ZR75-1:

The primers for B4GALT3 and GAPDH [table 4.2] as a normalising control gene were added to the master mix, where Z75-1 and HEK293 which acts as a calibrator, was considered as template, a qRT-PCR was performed.

Table 5.2 Gene expression analysis of B4GALT3 in ZR75-1 cell line:

Cell lines	CT value of B4GALT3		CT value of GAPDH	
ZR75-1	24.006	23.954	13.635	13.429
HEK293	24.994	24.854	17.416	16.276

Cell lines	Test experiment (TE)		Test Control (TC)		Housekeeping Experiment (HE)		Housekeeping Control (HC)	
ZR75-1	24.006	23.954	24.994	24.854	13.635	13.429	17.416	16.276

Cell lines	$\Delta\text{CT}(\text{TE}-\text{HE})$		$\Delta\text{CT}(\text{TC}-\text{HC})$		$\Delta\Delta\text{CT}(\Delta\text{CT TE} - \Delta\text{CT TC})$		$2\Delta\Delta\text{CT}(\text{fold change})$	
ZR75-1	10.371	10.525	7.578	8.578	2.793	1.946	0.1442	0.2594

Cell lines	Average of fold change	Standard deviation of fold change
ZR75-1	0.2018	0.0814

5.3.1.2 In MCF-7:

The primers for B4GALT3 and GAPDH [table 4.2] as a normalising control gene were added to the master mix, where MCF-7 and HEK293 which acts as a calibrator, was considered as template, a qRT-PCR was performed.

Table 5.3 Gene expression analysis of B4GALT3 in MCF-7 cell line:

Cell lines	CT value of B4GALT3		CT value of GAPDH	
MCF-7	24.963	26.729	24.986	21.856
HEK293	24.994	24.854	17.416	16.276

Cell lines	Test experiment. (TE)		Test Control (TC)		Housekeeping Experiment (HE)		Housekeeping Control (HC)	
MCF-7	24.963	26.729	24.994	24.854	24.986	21.856	17.416	16.276

Cell lines	Δ CT(TE-HE)		Δ CT(TC-HC)		$\Delta\Delta$ CT(Δ CT TE – Δ CT TC)		2 $\Delta\Delta$ CT(fold change)	
MCF-7	9.722	13.445	7.578	8.578	2.144	4.866	0.2262	0.0342

Cell lines	Average of fold change	Standard deviation of fold change
MCF-7	0.1302	0.0814

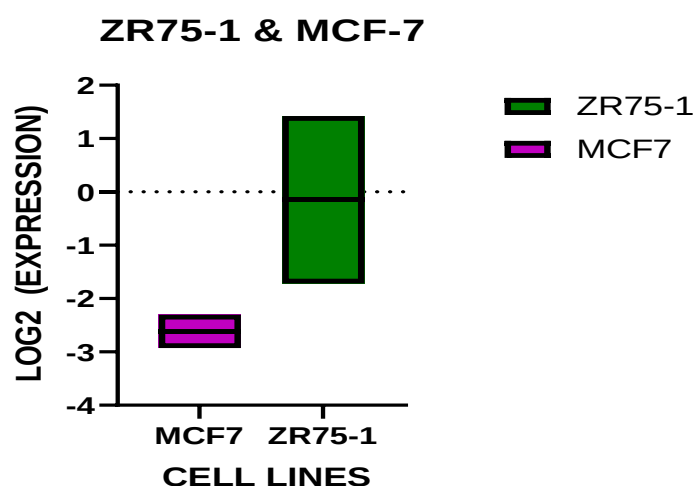


Fig 5.12 Graphical representation of the fold change expression of B4GALT3 gene in cell lines in logarithmic scale.

On comparison of the fold change expression in logarithmic scale of B4GALT3 in the cell lines, it was observed that the trend followed in the expression was similar to the expression Insilco analysis that was performed using GOBO [Fig 5.1]

5.3.2 Expression of B4GALT3 in cancerous tissue along with its corresponding paired normal:

The primers for B4GALT3 and GAPDH [table 4.2] as a normalising control gene were added to the master mix, where Breast Cancer Tumors and their corresponding paired normal, which acts as a calibrator, was considered as template, a qRT-PCR was performed.

Table 5.4 Gene expression analysis of B4GALT3 in Breast Cancer tumors and corresponding paired normal.

S.No	CT value of B4GALT3 of tumors		CT value of GAPDH	
1.	27.061	26.075	17.803	18.940
2.	27.520	28.231	18.820	19.155
3.	23.813	24.475	17.795	18.446
4.	28.748	28.348	20.275	20.201
5.	28.283	28.956	22.034	22.049
6.	27.364	26.951	16.762	17.736
7.	29.906	29.179	24.026	24.172
8.	23.968	23.841	17.318	17.711
9.	30.677	31.309	20.414	20.864
10.	27.225	26.806	18.920	18.765
11.	30.291	29.377	18.634	18.499
12.	27.150	26.982	21.556	21.710
13.	30.159	30.163	21.085	21.493
14.	26.123	26.946	18.570	19.372
15.	27.211	26.509	18.913	18.996

16.	35.517	34.502	28.483	27.424
17.	29.201	29.156	20.765	21.645

S.No	CT value of B4GALT3 of Paired Normal		CT value of GAPDH	
1.	28.636	29.391	23.083	22.632
2.	27.536	28.331	22.476	22.943
3.	26.826	26.532	20.989	21.227
4.	32.811	32.852	23.933	24.773
5.	28.958	29.107	22.619	22.862
6.	37.143	37.517	29.243	29.668
7.	30.712	31.479	24.714	24.405
8.	35.456	35.313	27.554	26.920
9.	29.431	30.309	24.185	24.084
10.	27.300	27.615	21.235	21.773
11.	30.623	30.935	22.069	22.515
12.	34.913	34.344	28.417	29.385
13.	33.988	35.494	27.128	27.340
14.	27.160	28.741	20.141	19.725
15.	30.981	33.671	24.743	24.646
16.	35.387	35.164	29.020	27.927
17.	35.610	35.713	24.424	24.425

S.no	Test experiment. (TE)		Test Control (TC)		Housekeeping Experiment (HE)		Housekeeping Control (HC)	
1.	27.061	26.075	28.636	29.391	17.803	18.940	23.083	22.632
2.	27.520	28.231	27.536	28.331	18.820	19.155	22.476	22.943
3.	23.813	24.475	26.826	26.532	17.795	18.446	20.989	21.227
4.	28.748	28.348	32.811	32.852	20.275	20.201	23.933	24.773

5.	28.283	28.956	28.958	29.107	22.034	22.049	22.619	22.862
6.	27.364	26.951	37.143	37.517	16.762	17.736	29.243	29.668
7.	29.906	29.179	30.712	31.479	24.026	24.172	24.714	24.405
8.	23.968	23.841	35.456	35.313	17.318	17.711	27.554	26.920
9.	30.677	31.309	29.431	30.309	20.414	20.864	24.185	24.084
10	27.225	26.806	27.300	27.615	18.920	18.765	21.235	21.773
11	30.291	29.377	30.623	30.935	18.634	18.499	22.069	22.515
12	27.150	26.982	34.913	34.344	21.556	21.710	28.417	29.385
13	30.159	30.163	33.988	35.494	21.085	21.493	27.128	27.340
14	26.123	26.946	27.160	28.741	18.570	19.372	20.141	19.725
15	27.211	26.509	30.981	33.671	18.913	18.996	24.743	24.646
16	35.517	34.502	35.387	35.164	28.483	27.424	29.020	27.927
17	29.201	29.156	35.610	35.713	20.765	21.645	24.424	24.425

S.NO	$\Delta CT(TE-HE)$		$\Delta CT(TC-HC)$		$\Delta \Delta CT(\Delta CT TE - \Delta CT TC)$		$2\Delta \Delta CT(\text{fold change})$	
1.	9.258	7.136	5.553	6.759	3.705	0.377	0.076685	0.770172
2.	8.700	9.076	5.059	5.387	3.641	3.689	0.080175	0.077557
3.	6.018	6.028	5.836	5.304	0.182	0.724	0.881273	0.605543
4.	8.472	8.147	8.877	8.080	-0.405	0.068	1.324265	0.954089
5.	6.249	6.908	6.340	6.245	-0.091	0.663	1.064907	0.631528
6.	10.601	9.215	7.900	7.850	2.701	1.365	0.153775	0.388135
7.	5.880	5.006	5.998	7.074	-0.118	-2.068	1.085235	4.191971
8.	6.651	6.129	7.902	8.393	-1.252	-2.263	2.381022	4.801023
9.	10.264	10.446	5.246	6.225	5.018	4.220	0.030866	0.053651
10.	8.305	8.041	6.065	5.843	2.240	2.198	0.211692	0.217876
11.	11.658	10.878	8.554	8.420	3.103	2.458	0.116347	0.182043
12.	5.594	5.272	6.497	4.960	-0.903	0.313	1.869486	0.805195
13.	9.074	8.670	6.860	8.154	2.214	0.516	0.215547	0.69924
14.	7.554	7.574	7.020	9.015	0.534	-1.441	0.690452	2.714641
15.	8.298	7.512	6.238	9.025	2.060	-1.513	0.239877	2.853521

16.	7.034	7.078	6.367	7.237	0.667	-0.159	0.629652	1.116677
17.	8.435	7.511	11.186	11.289	-2.751	-3.778	6.73113	13.71758

S.NO	Average	Standard Deviation
1.	0.423429	0.49037
2.	0.125821	0.08134
3.	0.743408	0.19497
4.	1.139177	0.261754
5.	0.848217	0.306445
6.	0.270955	0.165717
7.	3.005735	1.678485
8.	3.591023	1.711199
9.	0.042259	0.016111
10.	0.214784	0.004373
11.	0.149195	0.046454
12.	1.33734	0.752567
13.	0.457394	0.342022
14.	1.702546	1.431318
15.	1.546699	1.848125
16.	0.873165	0.344379
17.	10.22436	4.940166

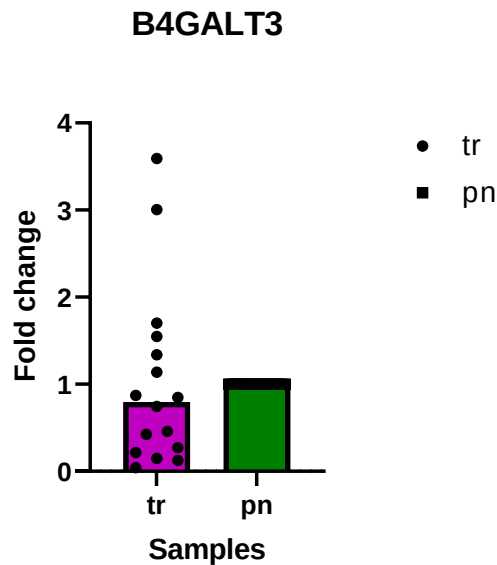


Fig 5.13 Graphical representation of the fold change expression of B4GALT3 gene in tumors and their corresponding paired normal.

On comparison of the fold change expression of B4GALT3 in the tumors and their corresponding paired normal, it was observed that the trend followed in the expression was similar to the expression Insilco analysis that was performed using UALCAN, on conclusion the expression of B4GALT3 was found to similar to that of the expression of the same in CPTAC samples. [Fig 5.3]

Calculation of Δ CT value:

The Δ CT value was calculated by normalising the CT value of the target gene in the tumor sample.

In order to calculate the Δ CT value of B4GALT3, Δ CT = ct value of targeted gene – ct value of control gene. For example, the subtraction of the CT value of GAPDH from the target gene CT value was found to be 9.258.

$$\Delta\text{CT} = 27.061 - 17.803 = 9.258$$

Calculation the $\Delta\Delta$ CT value.

The $\Delta\Delta$ CT is calculated by:

$$\Delta\Delta\text{CT} = \Delta\text{CT test experiment} - \text{average of the } \Delta\text{CT of the test control.}$$

For example, the value of the $\Delta\Delta CT$ Targeted B4GALT3 gene is 3.705.

which is calculated by,

$$\Delta\Delta CT = 9.258 - 5.553 = 3.705$$

Calculation of fold change

The fold change of the gene expression is calculated by:

$$\text{Fold change} = 2^{(-\Delta\Delta CT)}$$

For example, the fold change value of the B4GALT3 is calculated from $\Delta\Delta ct$ value is 0.076685.

$$\text{Fold change} = 2^{-(3.705)} = 0.076685.$$

Then the standard deviation of all the fold change was calculated, to calculate the gene expression values of <0.5 .

5.3.2.1 Highly expressed in tumor:

Few samples were found to be highly expressed in tumor than in paired normal. These samples indicate that expression of B4GALT3 is high in them compared to that of other samples.

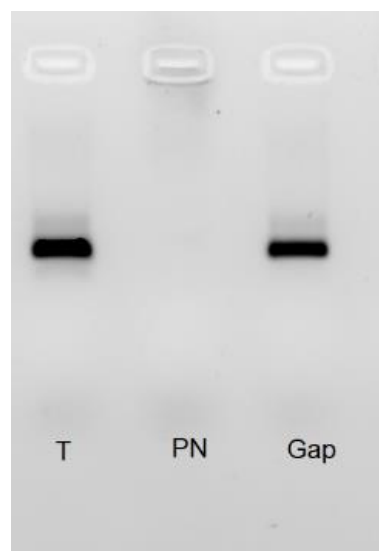


Fig 5.14 High expression of B4GALT3 in tumor and low expression of B4GALT3 in paired normal

5.3.2.2 Expression in paired normal compared to corresponding tumors:

In some samples, expression of B4GALT3 was found to be low than that of the corresponding paired normal.



Fig 5.15 B4GALT3 expressed in Paired Normal compared to expression in corresponding tumor.

5.3.2.3 Low or no expression of B4GALT3 in both cancerous tissues and corresponding paired normal:



Fig 5.16 No expression of B4GALT3 in both tumors and their corresponding paired normal.

In few samples, expression of B4GALT3 was found to be low in both, cancerous tissue and in their corresponding paired normal.

5.4 EXPRESSION ANALYSIS OF B4GALT4:

A qRT-PCR was performed with gene specific primers [table 4.2] for the gene B4GALT4, as well as GAPDH as a normalising control gene. By using the Promega GoTaq® qPCR Master Mix (Madison, Wisconsin, US) with respect to manufacturer's protocol in duplicates. The layout was planned in 96 well plate, where 10 µL of master mix, 1 µL of forward primer (10 µM), 1 µL of reverse primer (10 µM), 1 µL of cDNA template (50 ng) was prepared and was made up to 20 µL with sterile-nuclease free water were added. qRT-PCR amplification was conducted using the ABI Prism 7000 Sequence Detection System from Applied Biosystems, based in the USA. The thermal-cycling conditions includes 95°C for 2 min (polymerase activation), 95°C for 15 seconds, 60°C for 1 minute for 40 cycles.

5.4.1 EXPRESSION OF B4GALT4 IN CELL LINES:

Having the cell lines – ZR75-1 and MCF7 as template, qRT-PCR was performed, and the results were interpreted.

5.4.1.1 In ZR75-1:

The primers for B4GALT4 and GAPDH [table 4.2] as a normalising control gene were added to the master mix, where Z75-1 and HEK293 which acts as a calibrator, was considered as template, a qRT-PCR was performed.

Table 5.5 Gene expression analysis of B4GALT4 in ZR75-1 cell line:

Cell lines	CT value of B4GALT4		CT value of GAPDH	
ZR75-1	27.010	25.990	13.635	13.429
HEK293	25.383	25.088	17.416	16.276

Cell lines	Test experiment (TE)		Test Control (TC)		Housekeeping Experiment (HE)		Housekeeping Control (HC)	
ZR75-1	27.010	25.990	25.383	25.088	13.635	13.429	17.416	16.276

Cell lines	$\Delta\text{CT}(\text{TE-HE})$		$\Delta\text{CT}(\text{TC-HC})$		$\Delta\Delta\text{CT}(\Delta\text{CT TE} - \Delta\text{CT TC})$		$2\Delta\Delta\text{CT}(\text{fold change})$	
ZR75-1	13.375	12.561	7.967	8.812	5.408	3.750	0.0235	0.0743

Cell lines	Average of fold change	Standard deviation of fold change
ZR75-1	0.0489	0.0359

5.4.1.2 In MCF-7:

The primers for B4GALT4 and GAPDH [table 4.2] as a normalising control gene were added to the master mix, where MCF-7 and HEK293 which acts as a calibrator, was considered as template, a qRT-PCR was performed.

Table 5.6 Gene expression analysis of B4GALT4 in MCF-7 cell line:

Cell lines	CT value of B4GALT4		CT value of GAPDH	
MCF-7	24.986	21.856	15.241	13.284
HEK293	25.383	25.088	17.416	16.276

Cell lines	Test experiment (TE)		Test Control (TC)		Housekeeping Experiment (HE)		Housekeeping Control (HC)	
MCF-7	24.986	21.856	25.383	25.088	15.241	13.284	17.416	16.276

Cell lines	$\Delta\text{CT}(\text{TE-HE})$		$\Delta\text{CT}(\text{TC-HC})$		$\Delta\Delta\text{CT}(\Delta\text{CT TE} - \Delta\text{CT TC})$		$2\Delta\Delta\text{CT}(\text{fold change})$	
MCF-7	9.745	8.572	7.967	8.812	1.778	-0.240	0.2915	1.1811

Cell lines	Average of fold change	Standard deviation of fold change
MCF-7	0.736336	0.629049

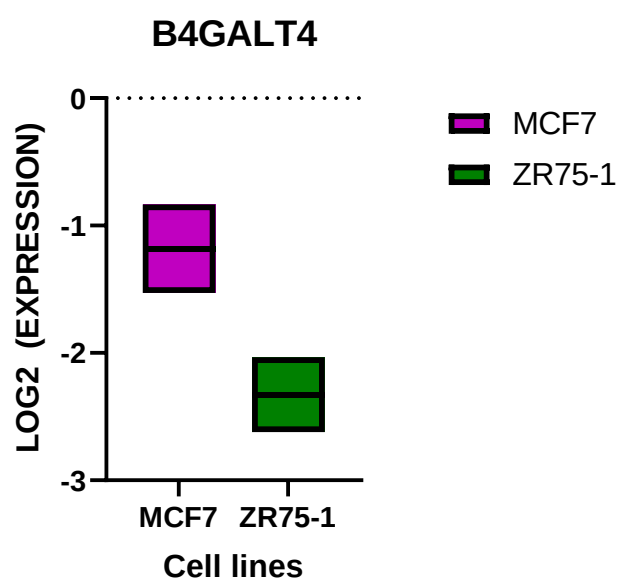


Fig 5.17 Graphical representation of the fold change expression of B4GALT4 gene in cell lines in logarithmic scale.

On comparison of the fold change expression in logarithmic scale of B4GALT4 in the cell lines, it was observed that the trend followed in the expression was similar to the expression Insilco analysis that was performed using GOBO [Fig 5.6]

5.4.2 Expression of B4GALT4 in cancerous tissue along with its corresponding paired normal:

The primers for B4GALT4 and GAPDH [table 4.2] as a normalising control gene were added to the master mix, where Breast Cancer Tumors and their corresponding paired normal, which acts as a calibrator, was considered as template, a qRT-PCR was performed.

Table 5.7 Gene expression analysis of B4GALT4 in Breast Cancer tumors and corresponding paired normal.

S.No	CT value of B4GALT4 of tumors		CT value of GAPDH	
1.	36.439	36.762	23.193	23.401
2.	33.083	32.665	18.835	18.804
3.	27.660	27.464	17.803	18.940
4.	29.462	28.817	18.820	19.155
5.	28.379	28.360	17.795	18.446
6.	27.569	28.228	20.275	20.201
7.	28.961	28.989	22.034	22.049
8.	29.378	29.673	23.284	26.633
9.	31.902	32.391	24.026	24.172
10.	26.547	25.970	17.318	17.711
11.	30.358	30.630	20.414	20.864
12.	28.066	28.390	18.920	18.765
13.	30.176	30.815	18.634	18.499
14.	34.056	33.374	27.575	27.759
15.	26.835	27.082	21.556	21.710
16.	28.543	27.955	18.913	18.996
17.	35.557	35.237	28.483	27.424
18.	25.901	24.699	14.644	16.747

S.No	CT value of B4GALT4 of Paired Normal		CT value of GAPDH	
1.	35.330	35.038	22.722	23.093

2.	40	40	23.820	23.979
3.	32.148	31.999	23.083	22.632
4.	31.050	30.971	22.476	22.943
5.	29.816	28.906	20.989	21.227
6.	32.876	33.115	23.933	24.773
7.	29.866	29.759	22.619	22.862
8.	31.734	32.040	24.714	24.405
9.	37.582	37.787	27.554	26.920
10.	32.676	32.804	24.185	24.084
11.	29.545	29.009	21.235	21.773
12.	32.273	32.131	22.069	22.515
13.	35.915	34.813	29.435	29.430
14.	34.615	35.709	28.417	29.385
15.	33.820	34.431	27.128	27.340
16.	29.599	34.073	24.743	24.646
17.	35.250	35.473	29.020	27.927
18.	38.000	37.525	28.171	28.228

S.no	Test experiment. (TE)		Test Control (TC)		Housekeeping Experiment (HE)		Housekeeping Control (HC)	
1.	36.439	36.762	35.330	35.038	23.193	23.401	22.722	23.093
2.	33.083	32.665	40	40	18.835	18.804	23.820	23.979
3.	27.660	27.464	32.148	31.999	17.803	18.940	23.083	22.632
4.	29.462	28.817	31.050	30.971	18.820	19.155	22.476	22.943
5.	28.379	28.360	29.816	28.906	17.795	18.446	20.989	21.227
6.	27.569	28.228	32.876	33.115	20.275	20.201	23.933	24.773
7.	28.961	28.989	29.866	29.759	22.034	22.049	22.619	22.862
8.	29.378	29.673	31.734	32.040	23.284	26.633	24.714	24.405
9.	31.902	32.391	37.582	37.787	24.026	24.172	27.554	26.920
10.	26.547	25.970	32.676	32.804	17.318	17.711	24.185	24.084
11.	30.358	30.630	29.545	29.009	20.414	20.864	21.235	21.773

12.	28.066	28.390	32.273	32.131	18.920	18.765	22.069	22.515
13.	30.176	30.815	35.915	34.813	18.634	18.499	29.435	29.430
14.	34.056	33.374	34.615	35.709	27.575	27.759	28.417	29.385
15.	26.835	27.082	33.820	34.431	21.556	21.710	27.128	27.340
16.	28.543	27.955	29.599	34.073	18.913	18.996	24.743	24.646
17.	35.557	35.237	35.250	35.473	28.483	27.424	29.020	27.927
18.	25.901	24.699	38.000	37.525	14.644	16.747	28.171	28.228

S.NO	$\Delta CT(TE-HE)$		$\Delta CT(TC-HC)$		$\Delta\Delta CT(\Delta CT TE - \Delta CT TC)$		$2\Delta\Delta CT(\text{fold change})$	
1.	13.246	13.361	12.608	11.945	0.639	1.416	0.6423	0.3748
2.	14.249	13.861	16.180	16.021	-1.931	-2.160	3.8130	4.4698
3.	9.857	8.524	9.065	9.367	0.792	-0.843	0.5776	1.7933
4.	10.642	9.662	8.573	8.028	2.069	1.634	0.2383	0.3220
5.	10.584	9.914	8.827	7.679	1.757	2.235	0.2957	0.2124
6.	7.293	8.027	8.943	8.342	-1.650	-0.315	3.1373	1.2436
7.	6.927	6.941	7.247	6.897	-0.320	0.044	1.2483	0.9700
8.	7.877	8.218	7.020	7.635	0.857	0.583	0.5520	0.6675
9.	9.229	8.258	10.029	10.867	-0.800	-2.609	1.7408	6.1004
10.	9.944	9.766	8.491	8.720	1.453	1.046	0.3651	0.4841
11.	9.146	9.625	8.310	7.236	0.836	2.389	0.5601	0.1909
12.	11.542	12.316	10.204	9.616	1.339	2.700	0.3953	0.1539
13.	6.481	5.615	6.480	5.383	0.001	0.233	0.9995	0.8511
14.	5.279	5.373	6.199	6.324	-0.920	-0.951	1.891	1.9335
15.	8.702	8.103	6.691	7.090	2.011	1.013	0.2481	0.4956
16.	9.629	8.958	4.856	9.427	4.774	-0.469	0.0365	1.3840
17.	7.074	7.813	6.230	7.546	0.843	0.267	0.5573	0.8310
18.	11.256	7.952	9.829	9.297	1.427	-1.345	0.3717	2.5404

S.NO	Average	Standard Deviation
1.	0.508568	0.189147

2.	4.141457	0.46437
3.	1.185496	0.859652
4.	0.280219	0.059217
5.	0.254108	0.058907
6.	2.190469	1.339043
7.	1.10921	0.19682
8.	0.609817	0.081639
9.	3.92062	3.082674
10.	0.424682	0.084162
11.	0.375574	0.261078
12.	0.576365	0.536355
13.	0.925355	0.104941
14.	1.912606	0.029688
15.	0.371914	0.17501
16.	0.710326	0.952846
17.	0.694197	0.193577
18.	1.456108	1.533466

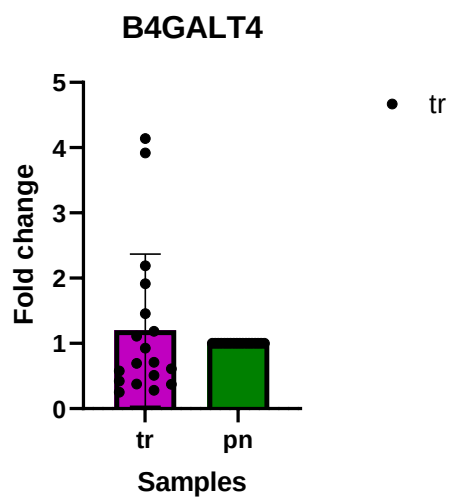


Fig 5.18 Graphical representation of the fold change expression of B4GALT4 gene in tumors and their corresponding paired normal.

On comparison of the fold change expression of B4GALT4 in the tumors and their corresponding paired normal, it was observed that the trend followed in the expression was similar to the expression Insilco analysis that was performed using UALCAN, on conclusion the expression of B4GALT4 was found to similar to that of the expression of the same in CPTAC samples. [Fig 5.8]

Calculation of Δ CT value:

The Δ CT value was calculated by normalising the CT value of the target gene in the tumor sample.

In order to calculate the Δ CT value of B4GALT4, Δ CT = ct value of targeted gene – ct value of control gene. For example, the subtraction of the CT value of GAPDH from the target gene CT value was found to be 13.246.

$$\Delta\text{CT} = 36.439 - 23.193 = 13.246.$$

Calculation the $\Delta\Delta$ CT value.

The $\Delta\Delta$ CT is calculated by:

$$\Delta\Delta\text{CT} = \Delta\text{CT test experiment} - \text{average of the } \Delta\text{CT of the test control}.$$

For example, the value of the $\Delta\Delta$ CT Targeted B4GALT4 gene is 0.639.

which is calculated by,

$$\Delta\Delta\text{CT} = 13.246 - 12.608 = 0.639$$

Calculation of fold change

The fold change of the gene expression is calculated by:

$$\text{Fold change} = 2^{(-\Delta\Delta\text{CT})}$$

For example, the fold change value of the B4GALT4 is calculated from $\Delta\Delta$ ct value is 0.076685.

$$\text{Fold change} = 2^{-(0.639)} = 0.6423.$$

Then the standard deviation of all the fold change was calculated, to calculate the gene expression values of <0.5 .

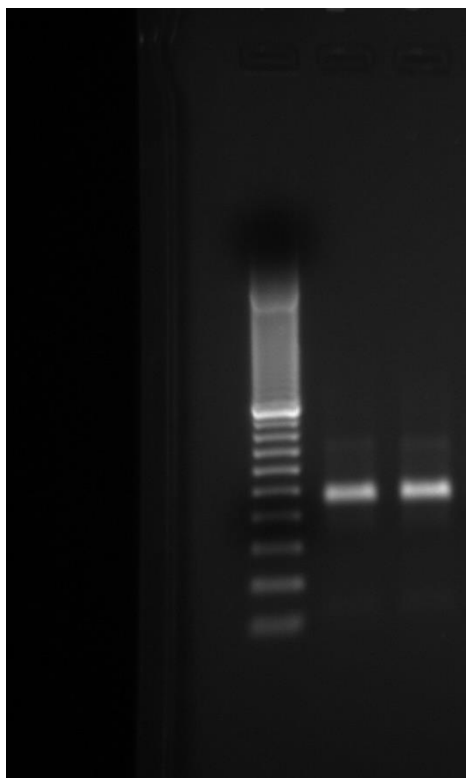


Fig 5.19 2% Agarose gel electrophoresis showing qRT-PCR amplification of B4GALT3 and B4GALT4 genes run along with DNA ladder.

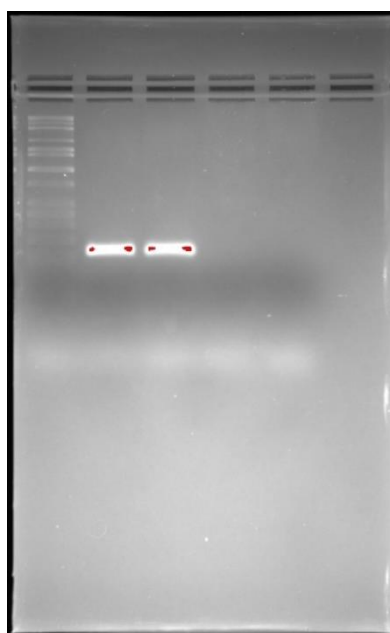


Fig 5.20 Expression of B4GALT3 and B4GALT4 in ZR75-1 cell line.

5.5 STATISTICAL ANALYSIS:

5.5.1 *B4GALT3* gene expression – statistical analysis:

An unpaired T-test was performed on the gene expression of B4GALT3 using GraphPad Prism Version 9.3.0. between tumors and the corresponding paired normal.

Unpaired t test

P value = 0.9098

P value summary - not significant

Significantly different ($P < 0.05$)? No

$t=0.1143$, $df=30$

5.5.2 *B4GALT4* gene expression – statistical analysis:

An unpaired T-test was performed on the gene expression of B4GALT4 using GraphPad Prism Version 9.3.0. between tumors and the corresponding paired normal

Unpaired t test

P value = 0.4660

P value summary – not significant

Significantly different ($P < 0.05$)? No

$t=0.7373$, $df=34$

CHAPTER 6

SUMMARY AND CONCLUSION

The expression levels of B4GALT3 and B4GALT4 genes were studied and analysed in Breast cancer cell lines and tissues by performing qRT-PCR. The studies conducted using Insilco analysis suggested that the expression of B4GALT3 gene was downregulated in the cell line MCF-7, when compared to the expression of the same in ZR75-1 cell line, on the other hand, B4GALT4 had more expression in MCF-7 cell line than in ZR75-1 [Fig 5.1 and Fig 5.6] . Similar trend was observed in the expression of B4GALT3 in MCF-7 and ZR75-1 [Fig 5.12 and Fig 5.13]. while for tumor samples, in TCGA datasets, tumors showed upregulation of B4GALT3 and B4GALT4 on comparison with its paired normal [Fig 5.2 and Fig 5.7], but the CPTAC analysis concluded the downregulation of B4GALT3 proteins [Fig 5.3] and for B4GALT4, a slight upregulation was observed. When Real Time PCR was performed to determine the expression of B4GALT3 and B4GALT4 in cell lines, tumors and their corresponding paired normal, a similar trend to that of CPTAC analysis was found. Based on the sample size, we found that B4GALT3 and B4GALT4 was downregulated and upregulated respectively in Breast Cancer tumors, the similar expression levels were also observed in the Breast Cancer cell lines.

A T-Test were performed and the significance of both the genes were calculated, for B4GALT3, the p value was found to $p = 0.9098$, on the other hand, for B4GALT4, the p value was found to be $p = 0.4660$. Both genes had p value more than 0.05, though there was a downregulation in B4GALT3, no significant difference in the expression was found. However, for B4GALT4, was found to be upregulated, there was no significant difference in the expression.

Further study in the expression of B4GALT3 and B4GALT4 has to be conducted to conclude the downregulation and upregulation of the genes respectively in the Breast Cancer.

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ANNEXURE

GATE ADMIT CARD



GRADUATE APTITUDE TEST IN ENGINEERING 2024
अभियांत्रिकी स्नातक अभिक्षमता परीक्षा २०२४
ORGANISING INSTITUTE: INDIAN INSTITUTE OF SCIENCE, BENGALURU
ADMIT CARD

Name of the Candidate **NITHYA SRI G**

Registration Number **BT24S57405205**

Paper Code/Name **BT : Biotechnology**

Date **10th February 2024 (Saturday)**

Time **9:30 AM to 12:30 PM**

Session **Forenoon : S5**

Photo ID Submitted **Aadhaar ID (Number : 782435874599)**



Nithya Sri G.

Examination Centre Code: 7405

Address of the Centre:

iON Digital Zone iDZ Manimangalam Center 3,
Dhanalakshmi College of Engineering,
Dr.V.P.R.Nagar,Manimangalam Village, Sriperumpuder Taluk,
Chennai, Tamilnadu, PIN : 601301, India.


Prof. Chandra Sekhar Seelamantula
Organising Chairperson, GATE 2024
(on behalf of NCB-GATE, for MoE)



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INSTRUCTIONS TO CANDIDATES

1. An electronic copy of the Admit Card is NOT acceptable. A printed copy of the Admit Card must be presented for verification along with the original valid photo identification document submitted at the time of application. In case the quality of the photograph on the identification document is poor, the candidate must bring an additional recent valid photo ID (Aadhaar Card, Driving License, PAN Card, Passport and Voter ID).

2. To facilitate the identity verification by the Examination Centre personnel, candidates are advised to report to the examination venue at least 90 minutes before the scheduled commencement of the examination. Candidates will NOT BE ALLOWED to login 30 minutes after the start of the exam.

3. Candidates will be permitted to occupy their seats before the scheduled start of the examination. Candidates can login and start reading the instructions 20 minutes before the start of the examination.

4. During the examination, a virtual scientific calculator will be available on the candidate's computer.

5. Personal calculators, mobile phones, watches of any kind or any other electronic devices are prohibited inside the Examination Centre. Charts, tables, papers, books, sheets and heavy ornaments are also not allowed inside the Test Centre.

6. A scribble pad will be provided for rough work. Candidates must write their name and Registration number on the scribble pad before using it. A candidate can possess only one scribble pad at any point of time. If the scribble pad gets filled, candidates can ask for another scribble pad, after returning the previous one to the

invigilator. A scribble pad once surrendered will not be returned to the candidate. At the end of the examination, the scribble pad in possession of the candidate must be returned to the invigilator.

7. Candidates must bring their own pen and pencil. Candidates are also allowed to bring a transparent water bottle.

8. Candidates are not allowed to bring any items other than those mentioned above. Examination Centre personnel are not responsible for the safety of the candidate's personal belongings. Candidates in possession of prohibited items will be deregistered, debarred from the examination, and may also be banned from appearing in future GATE examinations.

9. Candidates will not be allowed to leave the examination hall before the end of the examination.

10. Impersonation and violation of the above guidelines or the Code of Conduct as given in the **Information Brochure** will lead to cancellation of candidature and attract penal action.

11. PwD candidates are advised to bring their assistive devices, if any, and show them to the Examination Centre personnel for scrutiny.

12. PwD candidates who have been granted one-hour compensatory time in GATE 2024 examination are allowed to leave the examination hall after 12.30 PM in the forenoon session and after 5.30 PM in the afternoon session, by clicking the SUBMIT button only in the presence of the Invigilator. Once they click the SUBMIT button, the responses will be frozen, and the exam will end for them.

GATE SCORE CARD



GRADUATE APTITUDE TEST IN ENGINEERING 2024

अभियांत्रिकी स्नातक अभिक्षमता परीक्षा २०२४

ORGANISING INSTITUTE: INDIAN INSTITUTE OF SCIENCE, BENGALURU

SCORE CARD

Name of the Candidate

NITHYA SRI G

Name of the Parent/Guardian

G V HARI PRASAD

Registration No.

BT24S57405205

Test Paper

Biotechnology (BT)

Date of Examination

February 10, 2024

GATE Score

257

Marks out of 100

32.67

All India Rank (AIR)
in the test paper

5002

Qualifying Marks

General

38.9

EWS/OBC-NCL

35.0

SC/ST/PwD

25.9

Number of candidates
appeared for the test paper

17078



Nithya Sri G

Chandra Sekhar Seelamantula

Prof. Chandra Sekhar Seelamantula
Organising Chairperson, GATE 2024
On behalf of NCB-GATE
Ministry of Education (MoE)



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A candidate is considered **qualified** if the marks secured are greater than or equal to the qualifying marks mentioned for the category, for which a valid category certificate, if applicable, must be produced along with this Score Card.

This Score Card is valid
up to 31st March 2027.

GATE SCORE COMPUTATION

The GATE 2024 score is calculated using the formula

$$\text{GATE Score} = S_a + (S_t - S_a) \frac{(M - M_q)}{(M_t - M_q)}$$

where

M is the marks obtained by the candidate in the paper mentioned on the GATE 2024 Score Card

M_q is the qualifying marks for general category candidates in the paper

M_t is the mean of marks of top 0.1% or top 10 (whichever is larger) of all the candidates who appeared for the test paper

S_a = 350, is the score assigned to M_q

S_t = 900, is the score assigned to M_t

M_q is 25 marks (out of 100) or $\mu + \sigma$, whichever is greater. Here, μ is the mean and σ is the standard deviation of marks of all the candidates who appeared for the test paper.

Qualifying in GATE 2024 does not guarantee admission to a postgraduate program or scholarship/financial assistance. Admitting institutes may conduct additional tests or interviews for final selection of candidates.

Graduate Aptitude Test in Engineering (GATE) 2024 was organised by Indian Institute of Science, Bengaluru, on behalf of National Coordination Board (NCB) - GATE for the Department of Higher Education, Ministry of Education (MoE), Government of India.