

EXPRESSION ANALYSIS OF B4-GALT-I & B4-GALT-II 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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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade "A++" by NAAC

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APRIL - 2024



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This is to certify that this Project Report is the bonafide work of **JAYANTH. A (40230053)** who carried out Project work entitled **"Expression analysis of B4-GALT-I & B4-GALT-II in Breast cancer"** Under my supervision from October 2023 to March 2024.


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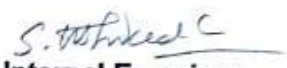
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ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to **Board of Management of Sathyabama Institute of Science and Technology** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr. V. RAMESH KUMAR, Ph.D.**, and Head of the Department of Biotechnology for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Internal Project Guide **Dr. INDUMATHI SM, Ph.D.**, for her constant support and encouragement during the advancement of my project work.

I am extremely grateful to my External Project Guide **Dr. G. GOPAL, Ph.D.**, for his valuable guidance, suggestions, and constant encouragement paved way for the successful completion of my Project work.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the completion of the project.

ABSTRACT

The Galactosyltransferase enzymes typically participate in the transfer of Galactose which is encoded by the GALT (Galactosyltransferase) Genes – these enzymes catalyse the transfer of galactose via linkages such as β 1-4, β 1-3, α 1-3 and α 1-4. In mammals this transfer is regulated by the B4GALT Genes. The B4GALT Family consist of seven such members, they are: B4GALT1, B4GALT2, B4GALT3, B4GALT4, B4GALT5, B4GALT6 and B4GALT7. In eukaryotes they are the located on the Endoplasmic Reticulum and Golgi Apparatus and are involved in the formation of several glycoconjugates and in lactose Biosynthesis. These Galactosyltransferases are a type of Trans-Golgi Glycosyltransferases (Glyco-T) that consist of type II transmembrane proteins, and are characterised by a short cytosolic segment, a single transmembrane domain of 20 amino acids and a catalytic domain.

In this study, the expression analysis of B4GALT1 and B4GALT2 genes was performed using Real time PCR, wherein the expression levels were first checked in the cell lines: MCF-7 and ZR75-1. The study a downregulation in the respective genes. Similarly, Expression analysis was also performed between tumor and pair normal samples, wherein, both B4GALT1 and B4GALT2 showed downregulation. This is conclusive that B4GALT1 and B4GALT2 act as tumor suppressors to an extent.

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LIST OF ABBREVIATIONS

B4GALT1	β - 4 Galactosyltransferase 1
B4GALT2	β - 4 Galactosyltransferase 2
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
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
HER2	Human epidermal growth factor receptor 2
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
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

CHAPTER - I

INTRODUCTION

1.1 BREAST CANCER

Breast cancer is effectively termed as the uncontrolled proliferation of cells within the breast tissue. It is the most diagnosed cancer and the leading cause of cancer-related mortality among women. Despite significant advances in research and clinical management, breast cancer continues to pose a formidable challenge to public health, due to various factors such as lifestyle, environment, and genetic complications.(Muss, 1996) At the molecular level, breast cancer is composed of various subtypes characterized by genetic alterations, gene expression profiles, and clinical behaviour. These subtypes include luminal A, luminal B, HER2-enriched, basal-like, and normal-like, each with distinct prognostic implications and therapeutic responses.(Boér, 2005)

While genetic predisposition plays a significant role in breast cancer susceptibility, with mutations in genes such as BRCA1 and BRCA2 conferring elevated risk, environmental and lifestyle factors also exert considerable influence.(Lerouge et al., 2004) Reproductive history, hormone exposure, obesity, alcohol consumption, and physical inactivity are among the modifiable risk factors implicated in breast cancer development, highlighting the importance of preventive strategies and lifestyle modifications in disease management.(Yang et al., 2005)

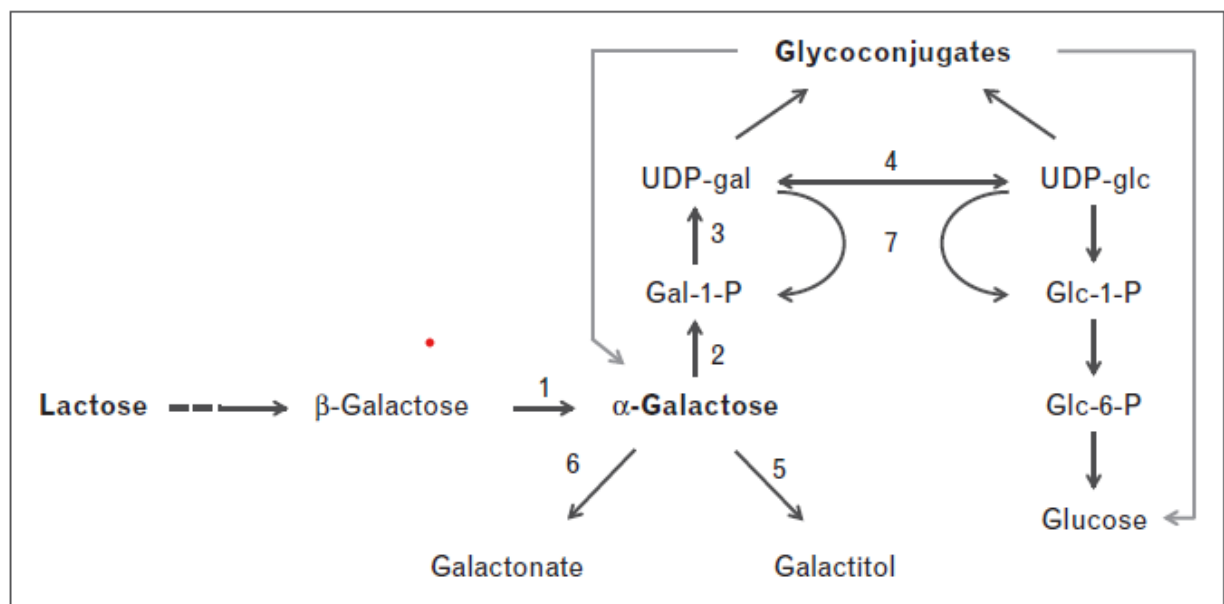
1.2 GALACTOSE METABOLISM

Galactose is an important carbohydrate for cellular metabolism and one of the most abundant sugars in the human diet. Historically and pan culturally, human diets have been primarily based on carbohydrates, accounting alone for 40–80% of the total daily calories.(Teixeira et al., 2024) The term carbohydrates derive from the definition “hydrates of carbon” which refers to polyhydroxylated compounds with a carbonyl group. The simplest form of carbohydrates is called monosaccharide. Two monosaccharides can be covalently linked together via a glycosidic bond to

generate a disaccharide, while a chain of multiple monosaccharide units is defined as oligosaccharide or polysaccharide.

Galactose metabolism involves enzymatic reactions, primarily in the liver, breaking down galactose for energy and biochemical pathways. Key steps include galactose conversion to galactose-1-phosphate by galactokinase, then to glucose-1-phosphate by Galactose-1-phosphate uridylyl transferase (GALT). This step is crucial, as GALT deficiencies cause galactosemia. Glucose-1-phosphate can be converted to UDP-glucose or glucose-6-phosphate. (Zangelmi et al., 2023)

Galactose metabolism is crucial for the breakdown and utilization of galactose, a sugar found in milk and dairy products. This process provides cells with energy and substrates for various biochemical pathways. Additionally, galactose metabolism is essential for maintaining glucose homeostasis in the body. Dysregulation of this process, as seen in conditions like galactosemia, can lead to serious metabolic disorders and health complications. Therefore, understanding and properly regulating galactose metabolism is vital for overall health and well-being. (Frey & Hegeman, 2013)



Galactose metabolism

Before β -galactose can enter the Leloir pathway, it must first be converted to the anomer by galactose mutarotase (GALM; enzyme 1)(Holden et al., 2003). In the Leloir pathway, α -galactose is first phosphorylated by galactokinase (GALK; enzyme 2) to galactose-1-phosphate (Gal-1-P). Galactose-1-phosphate uridylyl transferase (GALT, enzyme 3) then transfers the uridine monophosphate group (UMP) from UDP-glucose (UDP-glc) to Gal-1-P, which by glucose-1-phosphate (Glc-1-P) and UDP-galactose (UDP-gal)(Ross et al., 2004). In the third step of the Leloir pathway, UDP-galactose 4'-epimerase (GALE, enzyme 4) catalyzes the interconversion of UDP-gal and UDP-glc. Both UDP-gal and UDP-glc are sugar donors for glycosylation reactions and are important to produce complex carbohydrates. The formed Glc-1-P results in the production of glucose-6-phosphate (Glc-6-P) and glucose, thus resulting in energy production. As alternatives to the Leloir pathway, there are three minor pathways: conversion of galactose to galactitol by aldose reductase (enzyme 5), conversion of galactose to galactonate by galactose dehydrogenase (enzyme 6). Conversion of UDP-glucose/galactose -P to UDP-gal by pyrophosphorylase.(Szumilo, 1981)

1.3 GALT FAMILY

The GALT family refers to a group of enzymes involved in galactose metabolism, specifically in the conversion of galactose-1-phosphate to glucose-1-phosphate. This conversion is crucial for proper galactose utilization within cells(Daenzer et al., 2022). The most well-known enzyme in this family is galactose-1-phosphate uridylyl transferase (GALT), which catalyzes this reaction. The GALT family includes other related enzymes involved in similar biochemical reactions, such as UDP-glucose pyro phosphorylase, which converts glucose-1-phosphate to UDP-glucose. These enzymes collectively play critical roles in galactose metabolism and ensuring proper energy production and substrate utilization within cells.(Chatterjee et al., 2023)

These enzymes also play a crucial role in lactation and breast cancer through its involvement in galactose metabolism. During lactation, mammary glands produce and secrete milk, which contains lactose, a disaccharide composed of glucose and galactose. The mammary glands utilize the GALT family enzymes to metabolize

galactose derived from lactose for energy production and various cellular processes necessary for milk production and secretion but dysregulation or mutations in GALT family enzymes can impact lactation and breast health. For example, deficiencies in GALT activity, as seen in galactosemia, can lead to complications in infants, including poor feeding, failure to thrive, and liver damage.(Tatsumi et al., 2024)

In the context of breast cancer, research suggests a potential link between altered galactose metabolism and breast cancer development. Some studies have shown that abnormalities in galactose metabolism, including changes in GALT expression or activity, may contribute to breast cancer progression. Dysfunctional galactose metabolism can influence various cellular processes, such as cell proliferation, apoptosis, and signalling pathways involved in cancer development and progression.(Thoden et al., 1997)

Furthermore, alterations in lactose metabolism and galactose utilization may affect the tumor microenvironment and influence the availability of nutrients and energy sources for cancer cells. Understanding the role of the GALT family in lactation and its potential implications for breast cancer may provide insights into novel therapeutic targets and strategies for breast cancer prevention and treatment.(Abd-Elnaby et al., 2021)

1.4 ROLE OF GALT IN CANCER METABOLISM

The GALT family of proteins influences cancer by affecting galactose metabolism, glycolysis, the pentose phosphate pathway, epigenetic regulation, and interactions with oncogenic signalling pathways(L et al., 2020). Dysregulation of GALT activity in cancer cells alters energy production, biosynthesis, and cell growth, providing potential targets for therapeutic intervention in cancer treatment(Cramer et al., 1989).

These GALT family proteins, including galactose-1-phosphate uridylyl transferase, plays a key role in cancer cell metabolism by affecting galactose metabolism, glycolysis, the pentose phosphate pathway, epigenetic regulation, and interactions with oncogenic signalling pathways. Dysregulated GALT activity in cancer cells alters sugar metabolism and nutrient utilization, impacting energy production and biosynthetic pathways essential for cancer growth(Qin et al., 2005).

1.5 B4GALT1

B4GALT1 is a gene that encodes the enzyme beta-1,4-galactosyltransferase 1, that is integral to glycosylation processes responsible for cell signalling, and immune recognition. Dysregulated B4GALT1 contributes significantly to breast cancer pathogenesis, impacting tumor initiation, progression, and therapy resistance(De Vitis et al., 2019). Understanding B4GALT1's role in breast cancer biology is vital for developing targeted treatments and enhancing patient outcomes. B4GALT1 mediates glycosylation alterations affecting cellular processes, promoting tumor progression, immune evasion, and metastasis. Its expression varies across breast cancer subtypes and stages, correlating with aggressive phenotypes or favourable outcomes.(Staretz-Chacham et al., 2020)

Dysregulated B4GALT1 influences multiple cellular pathways crucial for tumor progression, including cell survival, migration, and immune modulation. Targeting B4GALT1 and associated glycosylation pathways holds promise for personalized breast cancer therapies, offering insights into disease mechanisms and therapeutic targets(Chen et al., 2023).

In the context of breast cancer, alterations in B4GALT1 expression levels have been observed in various studies, with both upregulation and downregulation reported in different subtypes and stages of the disease(Cui et al., 2023). High B4GALT1 expression has been associated with aggressive tumor phenotypes, including increased proliferation, invasion, and metastasis, as well as resistance to conventional chemotherapy and targeted therapies. Conversely, reduced B4GALT1 expression has been correlated with favourable clinical outcomes in some breast cancer subtypes, suggesting a context-dependent role in tumorigenesis and tumor progression(Wang et al., 2023).

1.6 B4GALT2

B4GALT2 is a gene that encodes for beta-1,4-galactosyltransferase 2, which is an enzyme involved in the glycosylation process, where sugar molecules are attached to proteins or lipids(Scott et al., 2016). In the context of breast cancer, B4GALT2 has gained attention due to its potential role in tumor progression and metastasis(Nuzziello et al., 2019). Studies have shown that B4GALT2 expression levels are elevated in breast cancer tissues compared to normal breast tissue, suggesting its involvement in disease development. Additionally, B4GALT2 has been linked to the modification of cell surface glycoproteins, which can impact cell adhesion, migration, and signalling pathways implicated in cancer progression.(Chatterjee et al., 2023)

Understanding the molecular mechanisms underlying B4GALT2's involvement in breast cancer may lead to the development of novel therapeutic strategies and biomarkers for early detection and prognosis. Elevated B4GALT2 levels correlate with advanced disease and poor prognosis, suggesting its potential as a diagnostic marker and therapeutic target in cancer. Understanding its role offers insights for targeting glycosylation pathways in cancer treatment.(Pai et al., 2021)

Elevated levels of B4GALT2 in cancer indicate dysregulated glycosylation processes within tumor cells, leading to increased aggressiveness, poor prognosis, altered immune response, these elevations in its levels can serve as biomarkers for cancer diagnosis, prognosis, and prediction of therapeutic response. Monitoring B4GALT2 expression levels may provide valuable information for patient management and treatment decisions(Muramoto et al., 2017).

1.7 Pathological role of B4GALT1 & B4GALT2

Beta-1,4-galactosyltransferase 1 (B4GALT1) and Beta-1,4-galactosyltransferase 2 (B4GALT2) are enzymes belonging to the B4GALT family, which catalyse the transfer of galactose from UDP-galactose to acceptor molecules, thereby forming β -1,4-glycosidic bonds. These enzymes play critical roles in glycosylation, a fundamental process in the modification of proteins and lipids, which influences various cellular functions, including cell-cell interactions, signal transduction, and protein stability. However, dysregulation or aberrant expression of B4GALT1 and B4GALT2 has been implicated in several pathological conditions, including cancer, immune disorders, and developmental abnormalities.

In cancer, alterations in B4GALT1 and B4GALT2 expression levels have been observed in various cancer types, and their pathological roles have been extensively studied. These enzymes contribute to cancer progression through multiple mechanisms such as glycosylation altering, promoting cancer adhesion, migration, invasion, and metastasis. These enzymes also modulate signalling pathways like Wnt/ β -catenin and Notch, enhancing cancer cell proliferation and survival. Additionally, they alter immune recognition by affecting glycosylation of cell surface antigens, contributing to tumor immune escape. Furthermore, B4GALT1 and B4GALT2-mediated glycosylation influence the tumor microenvironment, promoting a supportive niche for cancer growth, invasion, and metastasis.

CHAPTER - II

REVIEW OF LITERATURE

2.1 Breast Cancer: A survey

Breast cancer is one of the most common diseases worldwide. In 2020, there were 2.3 million women diagnosed with breast cancer and 685 000 deaths globally. As of the end of 2020, there were 7.8 million women alive who were diagnosed with breast cancer in the past 5 years, making it the world's most prevalent cancer. Breast cancer occurs in every country of the world in women at any age after puberty but with increasing rates in later life. (Taghian et al., 2006)

Breast cancer mortality changed little from the 1930s through to the 1970s when surgery alone was the primary mode of treatment (radical mastectomy) (McDonald et al., 2016). Improvements in survival began in the 1990s when countries established breast cancer early detection programmes that were linked to comprehensive treatment programs including effective medical therapies. Although the incidence of breast cancer remains high in the United States and Europe, both incidence and mortality have shown a decreasing trend in these countries. These trends are partially due to differences in receiving rates of screening mammography in these countries (Beslija et al., 2007).

Although screening mammography provides age-specific reductions in breast cancer mortality the receiving rate of mammography is decreasing overtime. Organized screening has reduced breast cancer mortality despite various substantial effects such as overdiagnosis, high cost, radiation exposure, and false positive biopsy recommendation (Zhang et al., 2021).

Various types of novel biomarkers in saliva have been reported for detecting breast cancer, such as epidermal growth factor (EGF), human epidermal growth factor receptor 2 (HER2), vascular endothelial growth factor (VEGF), carcinoembryonic antigen (CEA), cancer antigen 15-3 (CA15-3), and tumor suppressor oncogene protein. Recent technologies, such as transcriptomics, proteomics, and glycoproteomics, can simultaneously quantify hundreds of molecules and patterns to discriminate patients with breast cancer from healthy subjects (Yuan et al., 2021).

Metabolomics is a technology that enables profiling of metabolites and has the potential for screening of breast cancer]. Because it cannot quantify all metabolites by a single method, a limited number of molecules showing similar chemical properties, e.g., lipids, are profiled. Various analytical approaches are used in sample analysis, including nuclear magnetic resonance imaging and mass spectrometry (MS). Separation techniques, such as capillary electrophoresis (CE) or liquid chromatography (LC), are used before MS depending on the molecules of interest. There are two main approaches for MS-based proteomics. In bottom-up proteomics, the protein mixtures are digested and the resulting peptide mixtures are analyzed by liquid chromatography (LC)-MS and LC-tandem mass spectrometry (LCMS/ MS or shotgun approach) or separated by electrophoresis and then individual proteins are digested and analyzed by Matrix Assisted Laser Desorption/Ionization (MALDI)-MS in a method called peptide mass fingerprinting. In top-down proteomics, intact/whole proteins or a mixture of proteins are analyzed for molecular mass in MS mode and further fragmented to provide partial fragments in MS/MS mode.

2.2 Diagnostic Methods

2.2.1 Mammography

Mammography is an x-ray examination of the breasts that can reveal benign or malignant abnormalities. This is accomplished by compressing the chest post between two plates and applying a small amount of radiation to produce an x-ray image (Giampietro et al., 2020). Mammograms can be used for both screening and diagnosis. Mammography screening is performed to detect early signs of breast cancer before symptoms appear and reduce mortality through early diagnosis. Diagnostic mammography helps detect breast cancer when a woman experiences symptoms, such as a palpable lump in the breast.

In 2009, U.S. Preventive Services Task Force (USPSTF) issued new guidelines for mammography screening, recommending that women under 50 do not need routine mammography; This was consistent with the American Cancer Society guidelines,

which recommended ~2 mammograms every year for all women over 40 years. Additionally, breast density cannot be used to infer the information contained in her mammogram because the radiologist evaluates the information subjectively (Peisl et al., 2019).

For example, patients may have very different mammograms, each with very different results, but with the same breast density assessment. Previous studies used mammography results to generate statistics on glandular tissue volume. However, these automated methods for assessing breast density are not sufficient to predict breast cancer incidence. Recently, it was shown that gold-based nanoformulations can significantly improve the contrast of mammography images (Jang et al., 2019).

2.2.2 Magnetic Resonance Imaging

Breast MRI is a non-invasive, non-ionizing imaging tool that uses low-energy radiofrequency waves and magnetic fields to obtain detailed images of structures within the breast. MRI can be used to measure the size of cancer in women previously diagnosed with breast cancer and to look for metastatic tumors. Tumors smaller than 2 cm were accurately identified and measured using MRI (Muradali et al., 2023).

However, larger breast tumors are often overestimated due to abnormal breast tissue surrounding the actual lesion, which may lead to higher mastectomy rates. The American College of Radiology recommends several applications of breast MRI, including screening of high-risk populations (Gao et al., 2021). MRI can detect suspected breast malignancies that often evade clinical examination, mammography, and ultrasound. Fe₃O₄, gadolinium (III), and Mn (II)-based contrast agents are widely used for preoperative evaluation, especially for imaging the axillary lymph nodes of the breast.

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 type of non-contrast MRI that uses the diffusion of water molecules to create contrast in MR images, addressing some of the shortcomings of regular breast MRI.(Moeller et al., 2021) Potential benefits of DWI technology include improved differentiation between benign and malignant breast lesions, as well as evaluation and prediction of treatment efficacy. DWI enabled the identification of breast cancer, especially in dense breasts.(Partridge, 2020)

However, the sensitivity of DWI tends to differ compared to contrast-enhanced MRI. Technological innovations have helped overcome many of the image quality issues that have limited the widespread use of breast DWI. DWI has the potential to be an accurate, non-radioactive imaging modality, but it has not yet reached its full potential. Detailed investigations and clinical studies are currently required to demonstrate the ability of DWI to facilitate precise diagnosis of disease.

2.2.4 Positron Emission tomography (PET) Scanning and PET in Conjunction with Computer Aided Tomography (CT) Scanning (PET-CT)

Positron emission tomography (PET) imaging is widely accepted as an important clinical tool in oncology. Although many types of PET radiotracers have been developed to non-invasively study tumor metabolism in vivo, 2-deoxy-2-(18F) fluoro-D-glucose (FDG) is the most used. It is an FDA-approved PET radiotracer in use and utilizes. Enhanced glucose metabolism in cancer cells(Ikejiri et al., 2022). Cancer cells are highly proliferative and have a higher rate of glucose metabolism than normal cells. The FDG PET radiotracer enters cells via glucose transporters and is therefore taken up in greater amounts by tumor cells than by healthy cells. FDG intake is inversely associated with predictions(Bertagna et al., 2020).

PET-CT is a combination of PET (a nuclear medicine procedure) and CT that provides highly detailed images of the body. Improvements in the spatial resolution and sensitivity of breast PET (positron emission mammography) scanners have enabled clinical applications in the study of primary tumors. Many studies have shown that ^{18}F -FDG-PET/CT hybrid imaging provides information on cellular glucose uptake, which is increased in malignant lesions. Jorgensen et al. observed that the uptake of ^{18}F -FDG by tumor cells was significantly reduced after nanoparticle-assisted photothermal therapy, suggesting that it can be effectively used as a marker to assess treatment response. Physicians use PET-CT studies to diagnose and diagnose cancer, plan treatment, assess treatment effectiveness, and manage ongoing treatment (Ulaner et al., 2021).

2.3 Methodology for cancer classification

2.3.1 Filter approach

It evaluates features based on intrinsic properties of the data such as distance, correlation, and consistency, independently of the classifier. They are computationally intensive, have strong generalization ability, but have poor performance. Filter approaches are divided into univariate and multivariate. Univariate feature selection examines each feature individually to measure the strength of the association between the feature and the outcome variable. Common types are mutual information (MI) and information gain (IG). (Cree & Indave, 2020)

- MI measures the correlation between two variables.

In other words, it measures how much information one variable (X) knows about another variable (Y). Gene selection measures the correlation between genes and classification categories. The larger the MI value, the more gene information there is.

- Of the free site for psychological fortune-telling of animals as seen by the features that are strongly related to a class are those that have information, while unrelated features provide no information.(Yamato & Yoshida, 1982)

To determine the IG value, the entropy value, which represents the impurities of a given sample, is used. Then, a threshold is set and features with values greater than are selected. Multivariate evaluates features in other contexts. The most used techniques are:

- Minimum Redundancy and Maximum Relevance (mRMR) tends to select features that are highly correlated and poorly correlated with the class. It makes no sense to select both highly relevant and highly correlated features, as they do not add any further information due to their high correlation, but it increases the complexity of the model and makes overfitting more likely.

- a free site for psychological fortune-telling by animals.

- a free site for psychological fortune-telling by animals.

Features are evaluated according to the following hypotheses. “A good feature subset contains features that are highly correlated with classification but uncorrelated with each other.” In other words, features with low correlation with the class refer to irrelevant features, while informative features that are present are highly correlated(Rakha & Green, 2017).

- Fast correlation-based filter (FCBF) is a multivariate algorithm based on symmetric uncertainty (SU) that selects features that are highly correlated with a class.

Then, we apply heuristics to remove redundant features and retain the features that are relevant to the class.

2.4 Interactions between 4GAL-T1 and α - Lactalbumin (LA)

Of the seven known 4Gal-T members, only 4GalT1 is expressed at high levels in the mammary gland. It interacts with LA, an approximately 123-residue-long calcium-binding protein expressed only in the lactating mammary gland, to form the LS complex.(Yamato & Yoshida, 1982)

This complex transfers the galactose moiety of UDP-Gal to free glucose that does not function as an acceptor for 4Gal-T1, producing lactose (Gal1-4Glc), the major carbohydrate component of milk. The interaction of 4Gal-T1 with LA reduces the K_m for glucose by 1000-fold by promoting binding of Glc to the enzyme, thereby altering the specificity of the sugar acceptor.(Villagrán et al., 2019)

In the absence of LA, glucose has a K_m value of approximately 2 M. The interaction between 4Gal-T1 and LA is specific because lysozyme is a protein with a similar 3D structure and extensive sequence homology.It is noteworthy that the emergence of LA and LS systems in evolution is often associated with the emergence of mammals.

2.4.1 Structure of lactose synthase

The two proteins 4Gal-T1 and LA crystallize together as a complex (LS complex) only in the presence of either donor substrate or acceptor Glc or GlcNAc.

The crystal structure of LS in the presence of Glc or GlcNAc shows that LA interacts with 4Gal-T1 in a closed conformation at the site where the oligosaccharide binds to the enzyme, and Glc or GlcNAc in the monosaccharide binding pocket. It shows that it interacts with the enzyme(Pike et al., 1996). Therefore, LA competes with and interferes with the binding of one but not one oligosaccharide.(Picardo et al., 2019)

The fact that the closed conformation of 4Gal-T1 is induced only by substrate binding explains previous results shown by both precipitation analysis [104] and cross-linking experiments that the two proteins, interacts only in the presence of substrate. In the absence of substrate, 4Gal-T1 exists in an open conformation, and the residues involved in interaction with LA are buried under a long flexible loop, preventing interaction with LA. Therefore, in the absence of the substrate, the two molecules cannot cross-link. (Russo & Russo, 2006) Upon substrate binding, the long flexible loop of 4Gal-T1, the carboxyl-terminal residues from Arg359 to Ile363, forms a helix. The residues of the helix are rearranged and interact with both LA and GlcNAc. This explains why LA and 4Gal-T1 can only be cross-linked in the presence of the substrate. The interaction between LA and 4Gal-T1 in the closed conformation mostly involves the hydrophobic regions present in both molecules.

Residues Phe31 and His32 from the helix of LA and some of the residues in the carboxyl-terminal flexible region of LA that undergo helix-loop transition, residues 104–111, form a hydrophobic patch. 4Gal-T1 residues Phe360 and Ile363 form an extended region of hydrophobic patch and monosaccharide binding site together with residues Phe280, Tyr286, Gln288, and Tyr289 from the short helix of the long-loop closed structure.

2.4.1.1 Regulation of B4GALT in somatic cells

The transcriptional regulation of the murine β 1,4-galactosyltransferase (β 4-GT) gene, which encodes an enzyme involved in glycoconjugate biosynthesis as a housekeeping function in many tissues, but is also recruited for lactose biosynthesis specifically in the lactating mammary gland (Thompson et al., 2014). The β 4-GT gene contains three transcriptional start sites that are differentially used to produce 3.9 kb, 4.1 kb, and a germ cell-specific mRNA. Using DNase, I foot printing and electrophoretic mobility shift assays (EMSAs) with nuclear extracts from various tissues, the authors identify specific DNA binding sites and transcription factors involved in regulating expression from the two main start sites. They find that the region upstream of the 4.1 kb start site binds primarily the ubiquitous factor Sp1, regulating ubiquitous expression, while the region near the 3.9 kb start site binds mammary gland-specific factors AP2 and CTF/NF1 as well as Sp1 and a proposed

negative regulatory factor, achieving mammary gland-specific expression levels required for lactose production.

2.4.2 Functional analysis of *B4GALT1*

β -1,4-galactosyltransferase (β 4Gal-T1) is involved in the synthesis of the Gal β 1-4-GlcNAc disaccharide moiety of complex carbohydrates(De Vitis et al., 2019). It is a trans-Golgi glycosyltransferase (Glyco-T) with a type II membrane protein topology, a short N-terminal cytoplasmic domain, a transmembrane region, and a trans-Golgi compatible stem and C-terminal catalytic domain. Its hydrophobic transmembrane region, like other glyco-T proteins, is short compared to plasma membrane proteins, an important feature for its retention in the trans-Golgi apparatus. The catalytic domain has two flexible loops: a long loop and a small loop(Ru et al., 2023).

The primary metal binding site is located in the N-terminal hinge region of the long flexible loop. When metal ions and sugar nucleotides bind, the flexible loop undergoes a large conformational change from an open to a closed conformation. The conformational change simultaneously creates a previously absent oligosaccharide acceptor binding site in the C-terminal region of the flexible loop. The loop acts as a lid covering the bound donor substrate. After the transfer of the glycosyl moiety to the acceptor is complete, the sugar product is excreted. The loop returns to its original conformation, releasing the remaining nucleotide moiety(Y et al., 2021).

Structural changes in β 4Gal-T1 also create a binding site for the mammary gland-specific protein α -lactalbumin (LA), altering the enzyme's acceptor specificity for glucose to synthesize lactose during lactation.(Zhu et al., 2005) Sugar donor specificity is generally determined by several residues within the sugar nucleotide binding pocket of Glyco-T, and these residues are conserved among family members in different species. Mutation of these residues allows the design of novel glycosyltransferases with broader or desired donor and acceptor specificities and the synthesis of specific complex carbohydrates and specific inhibitors of these enzymes(Melin et al., 2019).

2.5 Functional analysis of glycosylation pathway genes such as B4GALT2

The molecular basis of aberrant glycosylation of proteins, a prevalent pathological change in colorectal cancers (CRCs), and the mechanisms by which it contributes to tumor progression remain largely unknown. Targeted resequencing of 430 glycosylation-related genes in patient-derived CRC cell lines (N=31) and matched primary tumor tissues, and identified 12 newly significantly mutated genes in colorectal cancer glycosylation-related genes were identified.

An enrichment of mutations in genes involved in the biosynthesis of N-linked and core 1-3 O-linked glycans in the colon (B3GNT2, B4GALT2, ST6GALNAC2), which accounted for approximately 16% of CRCs examined. Analysis of a large independent tumor tissue dataset identified recurrent mutations within these genes in colon and other gastrointestinal cancers.

Systematic biochemical and phenotypic characterization of candidate wild-type and mutant glycosyltransferases reveals that these mutations affect protein localization, post-translational modifications, encoded enzyme activity and/or colon cancer cell migration. It was shown to significantly change any of the functions.

These results suggest that functionally deleterious mutations in glycosyltransferase genes partially underlie glycosylation defects and that contributes to the pathogenesis of a molecular subset of colon and other gastrointestinal malignancies.

CHAPTER – III

3.1 AIM:

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

3.2 OBJECTIVES:

- To study the expression status of B4GALT-I and B4GALT-II in breast cancer cell lines.
- To compare the expression of the gene in breast cancer tissue using Real Time - PCR.
- To study the gene expression and survival status in other cancer subtypes.
- To perform in silico analysis of B4GALT I and B4GALT II.

CHAPTER - IV

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), in Dulbecco's modified Eagle's medium (DMEM) (HiMedia, India) or Roswell Park Memorial Institute - 1640 (RPMI-1640) (Himedia, India), supplemented with 10% Fetal bovine serum (FBS) (GIBCO, USA) containing 100 mg/L penicillin and 100 mg/L streptomycin (HiMedia, India) at 37°C and 5% CO₂ in a humidified incubator. For subculture, 80–90% confluent cells were washed in sterile phosphate-buffered saline (PBS), followed by the addition of 0.5–2 mL of trypsin-EDTA solution [0.25% w/v trypsin (Sigma Aldrich, India) and ethylene diamine tetraacetate (EDTA) (MB biomedical, India) 0.001% w/v dissolved in PBS and sterilized with 0.2 µm filter]. Cells were then centrifuged at 800 rpm for 2 min and the pellet was suspended in growth medium containing 10% FBS to inactivate trypsin 10 µl of cell suspension was stained with 10 µl of 0.4% trypan blue solution and cell number was estimated using a Countess II FL automatic cell counter (Thermo Scientific, USA). Cell line stocks were frozen in liquid nitrogen using a freezing mixture consisting of 70% FBS, 20% DMEM, and 10% dimethyl sulfoxide.

Table 4.1 List of cell lines used

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

4.2 RNA isolation

The total RNA was isolated from the tissues using Trizol (Sigma-Aldrich, USA). The tissues were first frozen using liq-N₂. The tissue was then ground into fine particles using a mortar and pestle. These granules were then suspended in Trizol (Sigma-Aldrich, USA) for 15-20 minutes. It was then centrifuged at 10,000 rpm for 3 min at 4 °C. The supernatant was collected into a new centrifuge tube (Eppendorf, USA) and the pellet was discarded. One-tenth of the total volume was added to the tissue suspended in Trizol, mixed gently, and incubated for 5 min at room temperature. The solution was then centrifuged at 10,000 rpm for 15 min at 4 °C. The aqueous phase was pipetted off without disturbing the other layers, transferred to a new 1.5 ml tube, and placed on ice. An equal volume of isopropanol was added to the aqueous phase. Mixed gently and then incubated for 10 minutes. The samples were then centrifuged at 4 °C and 10,000 rpm for 20 min. The resulting white pellet was washed twice with 75% ethanol. The pellet was then air-dried and dissolved in 15 µl of sterile nuclease-free water. The RNA concentration was then quantified using a Nanodrop ND 2000 spectrophotometer (Thermo Scientific, USA). Nuclease-free sterile water was used for blank measurements, and 1 µl of sample was used to estimate RNA concentration in ng/µl. An absorbance ratio of 1.8 to 2 at 260 nm/280 nm indicated the purity of RNA isolated from the samples.

4.3 Agarose Gel Electrophoresis

Agarose gel electrophoresis was performed to confirm RNA integrity. 100 ml of 0.8% agarose (Cytvia, USA) in 1× Tris/borate/EDTA (TBE) buffer (0.13 M Tris, 5 mM boric acid and 2.5 mM EDTA, pH 7.6) was prepared. The agarose was heated in the oven until completely dissolved, poured into a gel dish, and placed with a comb. Then the agarose was allowed to solidify for 30 min, the gel dish was immersed in a gel tank containing 1x TBE buffer 1 µg of total RNA was mixed with 6x transparent safety dye (Green Biosciences, India), loaded onto an agarose gel, and run at 100 V for 30 min. Gels were visualized and documented using ChemiDoc™ MP imaging system “Biorad, USA”. Under UV absorbance.

4.4 cDNA Conversion

1 µg of total RNA was converted to cDNA using the PrimeScript™ RT-PCR kit (DSS Takara Clontech, Japan) in a two-step procedure according to the manufacturer's protocol. 1 µg of total RNA, 1 µl of oligo DT primer (2.5 µM), 1 µl of dNTP mixture (10 mM) were added, and the volume was adjusted to 10 µl with sterile nuclease-free water. The tubes were placed in a thermal cycler for denaturation and annealing at 65 °C for 5 min, followed by 4 °C for 10 min. The above reaction mixture was then added to 4 µl 5X PrimeScript buffer, 0.5 µl RNase inhibitor (40 U/µl), and 0.5 µl reverse transcriptase and made up to 20 µl with sterile 52 nuclease-free water. Place the tube in a thermocycler and cycle conditions are 30 °C for 5 min, 42 °C for 30 min, 95 °C for 5 min, and 4 °C.

4.5 Designing of Real-Time q-PCR primers

Primers for the 2 B4GALT genes, House Keeping genes are listed in Table 4.2. These were designed using the Primer Design Tool from Integrated DNA Technologies (Coralville, Iowa, USA). Primers were validated by Primer BLAST (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>) and submitted to Eurogentec in Belgium for synthesis. Primers were dissolved in nuclease-free sterile water and diluted to a working concentration of 10 µM for further use.

Table 4.2 List of primers used for q-RT-PCR

Gene	Primers for qRT-PCR
B4GALT1	FP: 5'GTATTTTGGAGGTGTCTCGCTC3' RP: 5'GGGCGAGATATAGACATGCCTC3'
B4GALT2	FP: 5'GACCGCGACAAGCATAACGAAC3' RP: 5'AGACACCTCCAAGACCTGGTAC3'
GAPDH	FP: 5'GTCTCCTCTGACTTCAACAGCG3' RP: 5'ACCACCCTGTTGCTGTAGCCAA3'

4.6 Quantitative real-time -PCR (qRT-PCR)

qRT-PCR was carried out with the gene-specific primers for the two B4GALT genes (B4GALT1 and B4GALT2) and GAPDH as the normalising control genes, by utilizing the SensiFAST™ SYBR® Lo-ROX Kit (Bioline, UK) with respect to manufacturer's protocol in the form of 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 include: (Hold Stage: 50°C for 2 min, 95°C for 2 min) and (PCR Stage: 95°C for 15 sec and 60°C for 1 min) for 40 cycles.

Then 96 -well plate containing the reaction mixture was subsequently analyzed using Quantstudio12K real-time PCR (Thermo Fischer Scientific, USA). The program was set up as a standard 96-well plate to calculate cycle threshold (CT) values and 40 cycles. Relative expression levels between target and control were determined using the $2^{-\Delta\Delta CT}$ method. This method assumes that the relative gene expression of the reference sample is 1, so 2^0 has a value of 1. 100% efficiency and uniform PCR amplification across all samples is assumed. For B4GALT gene expression analysis in cancer cell lines, HEK-293, MCF-7 and ZR-751 were used as control cell lines.

4.7 Breast tissue sample preparation

Tissue samples were collected after obtaining informed consent from breast cancer patients who underwent modified radical mastectomy without prior treatment. Tissue adjacent to the tumor was collected, histologically determined to be normal cells, and designated and used as the normal paired (PN) sample. total, 19/36 patients were diagnosed with invasive ductal carcinoma, 8/36 patients were diagnosed with invasive breast cancer, and 3/36 patients were diagnosed with ductal carcinoma in situ (DCIS). and 3/36 patients were diagnosed with mucosal cancer. One patient was diagnosed with metaplastic (spindle cell) carcinoma, one

patient was diagnosed with poorly differentiated malignancy, and one patient was diagnosed with invasive breast cancer with cellular mucin and pseudo signet ring cells. The tissues were then collected and stored in RNA Later for 24 hours to preserve the RNA. The RNA Later was then removed and the samples were frozen at -80°C .

4.8 Statistical analysis

GraphPad Prism software (version 9, USA) was used to perform an unpaired t-test. The p -values of less than 0.05 were considered as significant. The values were represented as mean $\pm$ SD.

CHAPTER – V

RESULTS AND DISCUSSION

5.1 In-Silico Analysis

5.1.1 B4GALT1

5.1.1.1 Cell Line Expression

The expression of B4GALT1 was analysed for various cell lines from the GOBO Dataset. The analysis showed slight upregulation in cell lines such as: BT20, SUM225, HCC1954, HS578T, MDAMB415, SUM52PE, T47D, MCF7, X600MPE and BT474. While prominent upregulation was seen in: HCC3153, HCC1569, HCC2157, HCC1143, MDAMB157, MDAMB436, BT549, HBL100, HCC202, SKBR3, HCC2185, LY2. Downregulation was observed in cell lines such as: MDAMB468, HCC1937, HCC1187, MCF12A, HCC1500, MDAMB231, MCF10A, MDAMB134VI, ZR751, SUM44PE, HCC1428, CAMA1, BT483, AU565, MDAMB175VII, ZR75B, HCC1007, UACC812, SUM185PE and ZR7530. Whereas, cell lines such as: HC70, HCC38 and SUM159PT, showed no significant expression.

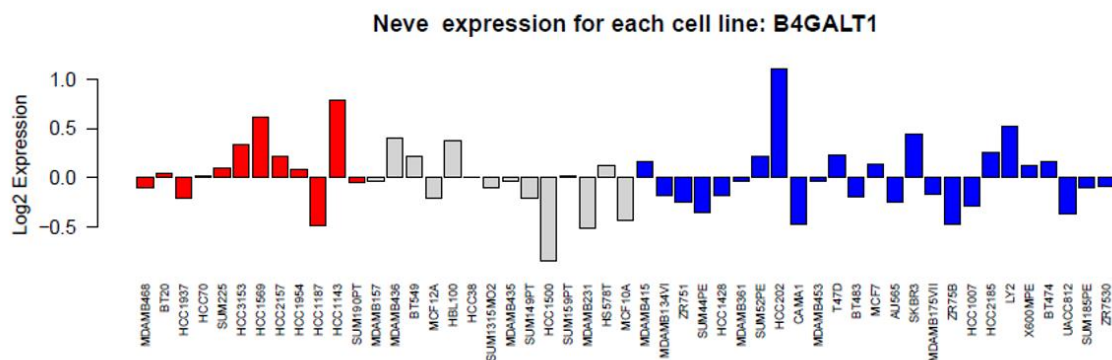


Fig 5.1: Expression of B4GALT1 in different cell lines: Depicts the expression of B4GALT1 in different cell lines on a logarithmic scale amongst various cell lines.

5.1.1.2 B4GALT1 Expression in cancers

B4GALT1 gene expression was analysed using the UALCAN TCGA datasets. The analysis showed that B4GALT1 had an upregulation in Bladder Urothelial carcinoma (BLCA), Breast invasive carcinoma (BRCA), Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), Cholangio carcinoma (CHOL), Esophageal carcinoma (ESCA), Head and neck squamous cell carcinoma (HNSC), Lung adenocarcinoma (LUAD), Sarcomatoid carcinoma (SARC), Stomach adenocarcinoma (STAD) and Uterine corpus endometrial carcinoma (UCEC).

Whereas, Downregulation was observed in Colon adenocarcinoma (COAD), Kidney chromophobe (KICH), Kidney renal clear cell carcinoma (KIRC), Kidney renal papillary cell carcinoma (KIRP), Pancreatic adenocarcinoma (PAAD), Prostate adenocarcinoma (PRAD), Pheochromocytoma and paraganglioma (PCPG), Human skin cutaneous melanoma (SKCM) and Thyroid carcinoma (THCA).

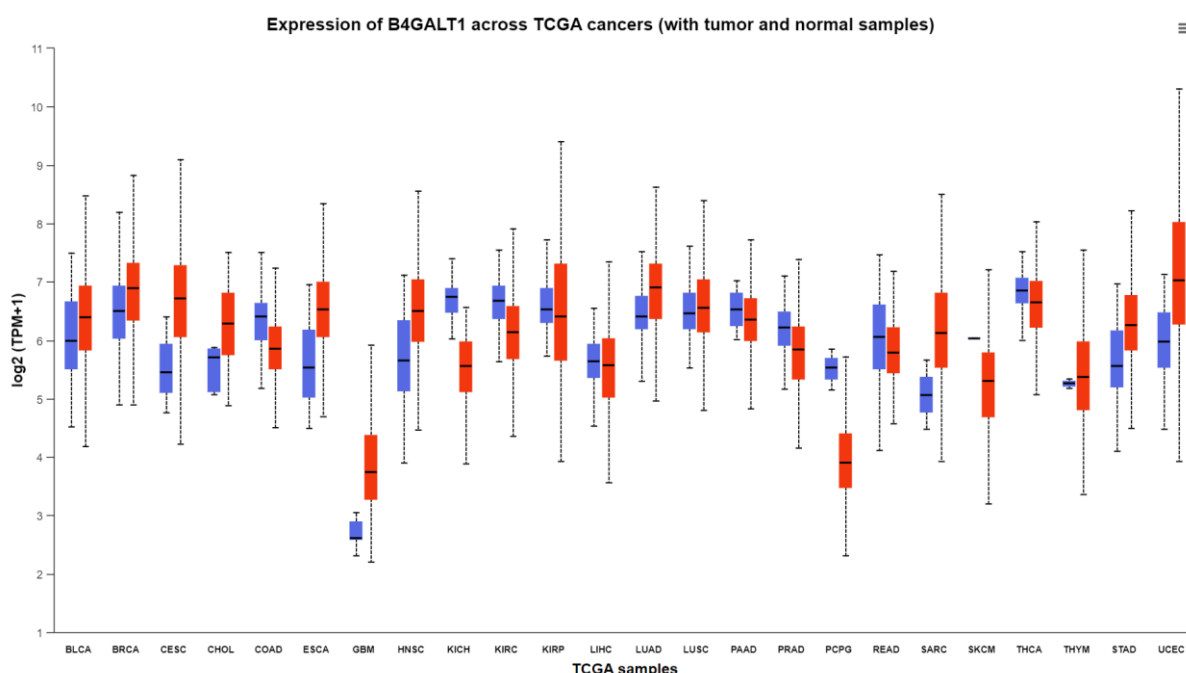


Fig 5.2: Expression of B4GALT1 in different cancers: The graph depicts the upregulation and downregulation of B4GALT1 in different cancers.

5.1.1.3 Gene-set and multivariate analysis of B4GALT1

This data on the expression of B4GALT1 was plot using the GEPIA database. (A) represents the survival rate of patients with respect to the expression levels of B4GALT1, (B) depicts the expression levels with reference to different cell lines, (C) co-relates the Hazard ratio with respect to the different classifying parameters of cancer. (D) indicative of multivariate analysis.

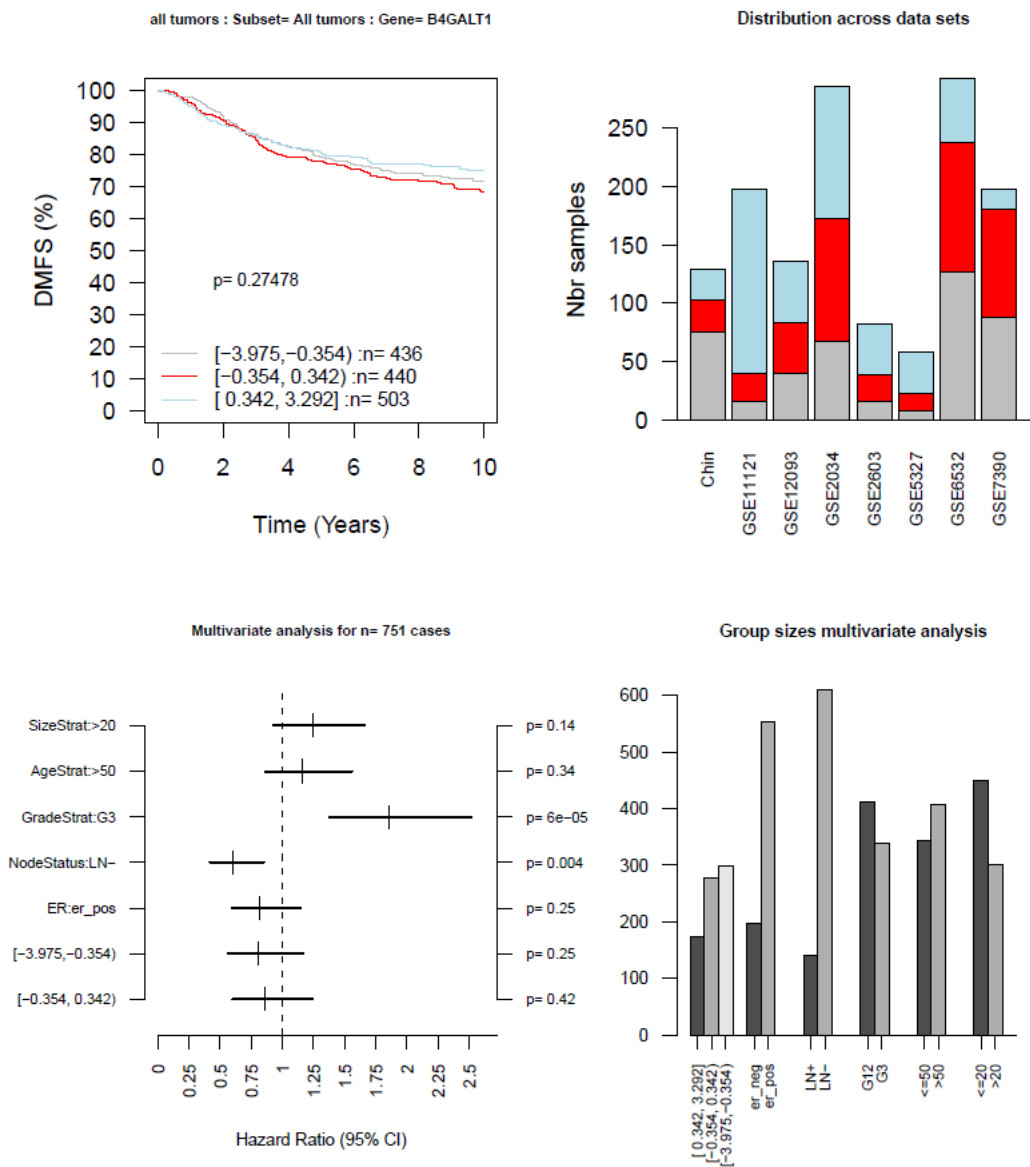


Fig 5.3: multivariate expression analysis of B4GALT1

5.1.1.4 Proteomic expression of B4GALT1 in cancers

The proteomic expression of B4GALT1 was analysed in various other cancers using the UALCAN CPTAC dataset. The analysis showed upregulation of B4GALT1 in Colon Cancer, Clear cell RCC, Uterine corpus endometrial carcinoma (UCEC), Lung cancer, Pancreatic adenocarcinoma (PAAD), Glioblastoma and Liver cancer.

Whereas, downregulation was observed in Breast cancer alone, alongside similar expression levels in head and neck carcinoma.

This analysis approach compares the expression levels of B4GALT1 between tumor and Pair normal tissues for each of the mentioned cancer types. This analysis was depicted using the Box whisker plot.

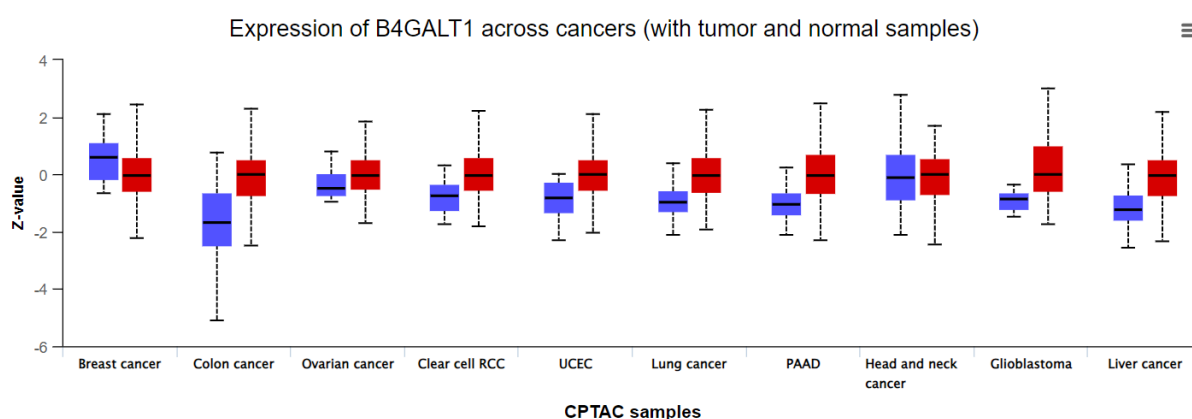


Fig. 5.4 Expression of B4GALT1 in cancer as CPTAC: The graph depicts the proteomic expression of B4GALT1 between the tumors and pair normal across various cancers.

5.1.1.5 Validation of B4GALT1 expression in cancer by Boxplot analysis of GEPIA

The gene expression of the B4GALT1 was also analyzed by Boxplot analysis of TCGA datasets in GEPIA. It was observed that the expression of B4GALT1 was upregulated in tumors, compared to the regulation in the pair normals. The results of the gene expression analysis of B4GALT1 by using the TCGA dataset has shown considerably different levels of regulation. Since the gene expression was varied, the protein expression was evaluated.

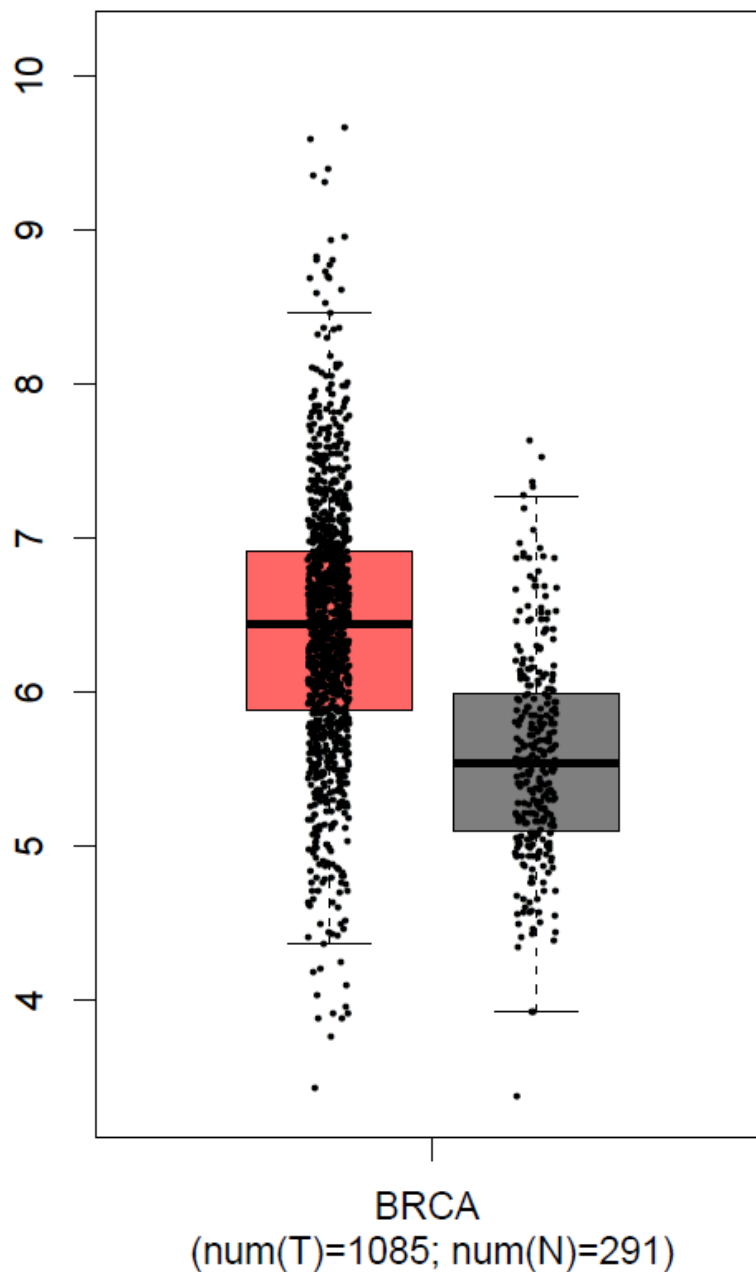


Fig 5.5 B4GALT1 expression validation by boxplot

5.1.1.6 B4GALT1 survival analysis in breast cancer

Survival analysis using GEPIA datasets was performed for the gene B4GALT1. It was inferred from the Hazard Ratio (HR) = 0.51, that there is no significant effect between the expression levels of B4GALT1 and the survival rate of patients.

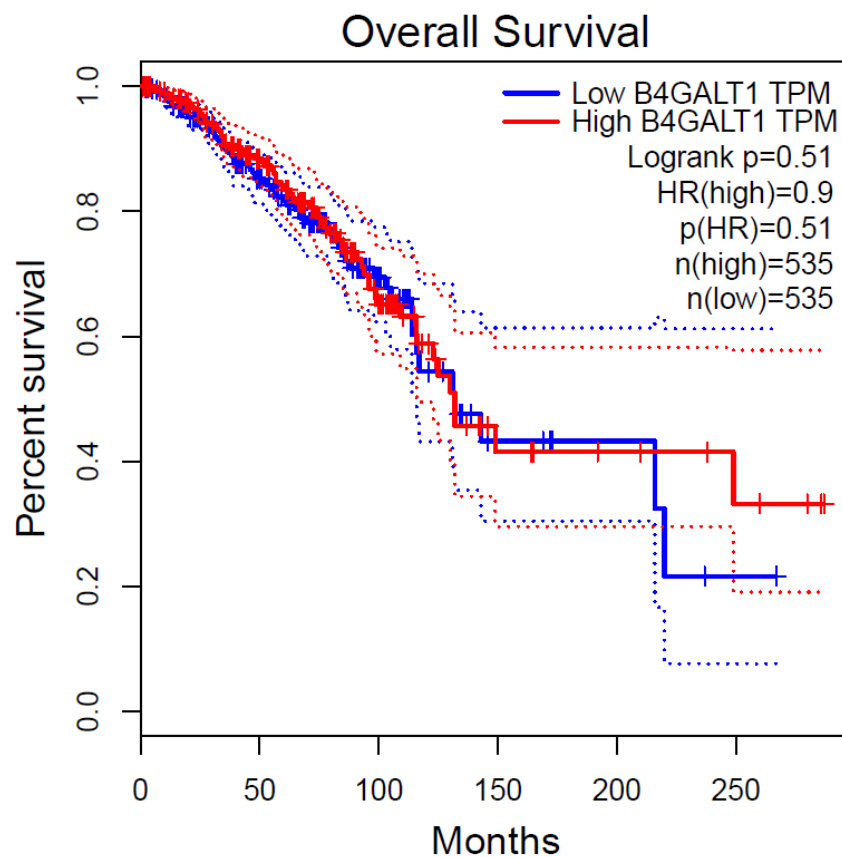


Fig 5.6 Survival analysis for Breast cancer

5.1.2 B4GALT2

5.1.2.1 Cell line expression

The expression of B4GALT1 was analysed for various cell lines from the GOBO Dataset. The analysis showed slight upregulation in cell lines such as: HCC1954, MDAMB157, MDAMB415, MDAMB134VI, HCC202, ZR75B, UACC812 and SLM185PE. While prominent upregulation was seen in: HCC70, HCC1569, HCC2157, HCC1187, BT549, MCF12A, HCC38, SUM1315MO2, SUM149PT, HCC1500SUM159PT, HS578T, MCF10A, AU565 and SKBR3. Downregulation was observed in cell lines such as: BT20, HCC1937, SUM225, HCC1143, SUM190PT, MDAMB436, HBL100, MDAMB435, MDAMB231, ZR751, SUM44PE, HCC1428, MDAMB361, SUM52PE, CAMA1, MDAMB453, T47D, MCF7, MDAMB175VII, HCC2185, LY2, X600MPE and BT474. Whereas, cell lines such as: HCC3153, BT483, HCC1007 and ZR7530, showed no significant expression.

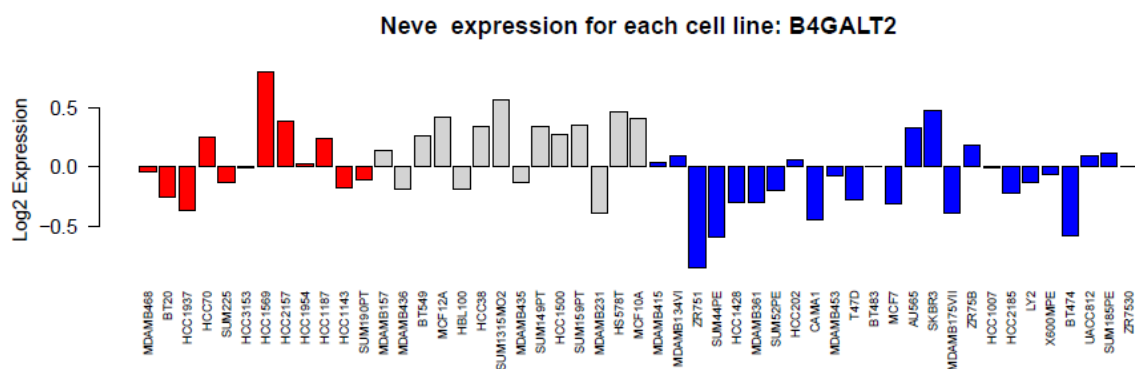


Fig 5.7: Expression of B4GALT2 in different cell lines: Depicts the expression of B4GALT2 in different cell lines on a logarithmic scale amongst various cell lines.

5.1.2.2 B4GALT2 expression in cancers

B4GALT2 gene expression was analysed using the UALCAN TCGA datasets. The analysis showed that B4GALT2 had an upregulation in Bladder Urothelial carcinoma (BLCA), Breast invasive carcinoma (BRCA), Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), Cholangio carcinoma (CHOL), Colon adenocarcinoma (COAD), Esophageal carcinoma (ESCA), Head and neck squamous cell carcinoma (HNSC), Liver hepatocellular carcinoma (LIHC), Lung adenocarcinoma (LUAD), Lung squamous cell carcinoma (LUSC), Pheochromocytoma and paraganglioma (PCPG), Rectum Adenocarcinoma (READ), Sarcomatoid carcinoma (SARC), Human skin cutaneous melanoma (SKCM), Thyroid carcinoma (THCA), Stomach adenocarcinoma (STAD) and Uterine corpus endometrial carcinoma (UCEC).

Whereas, Downregulation was observed in Kidney chromophobe (KICH), , Pancreatic adenocarcinoma (PAAD) and Thymic carcinoma (THYM).

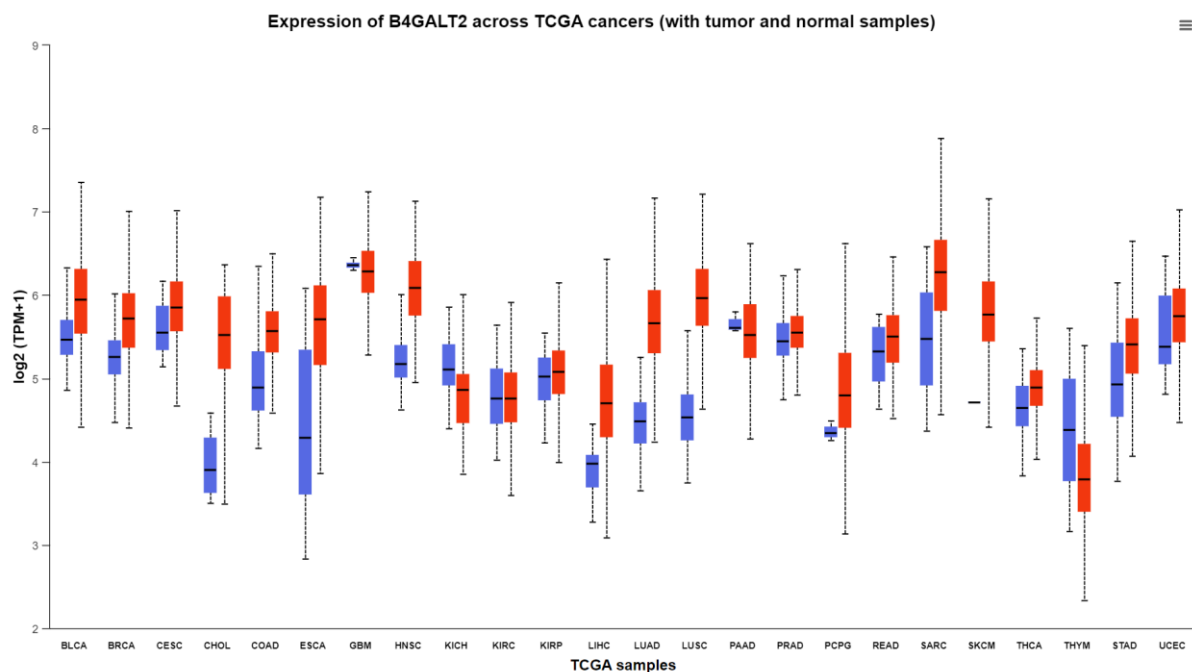


Fig 5.8: Expression of B4GALT2 in different cancers: The graph depicts the upregulation and downregulation of B4GALT2 in different cancers.

5.1.2.3 Gene-set and Multivariate analysis of B4GALT2

This data on the expression of B4GALT2 was plot using the GEPIA database. (A) represents the survival rate of patients with respect to the expression levels of B4GALT2, (B) depicts the expression levels with reference to different cell lines, (C) co-relates the Hazard ratio with respect to the different classifying parameters of cancer. (D) indicative of multivariate analysis.

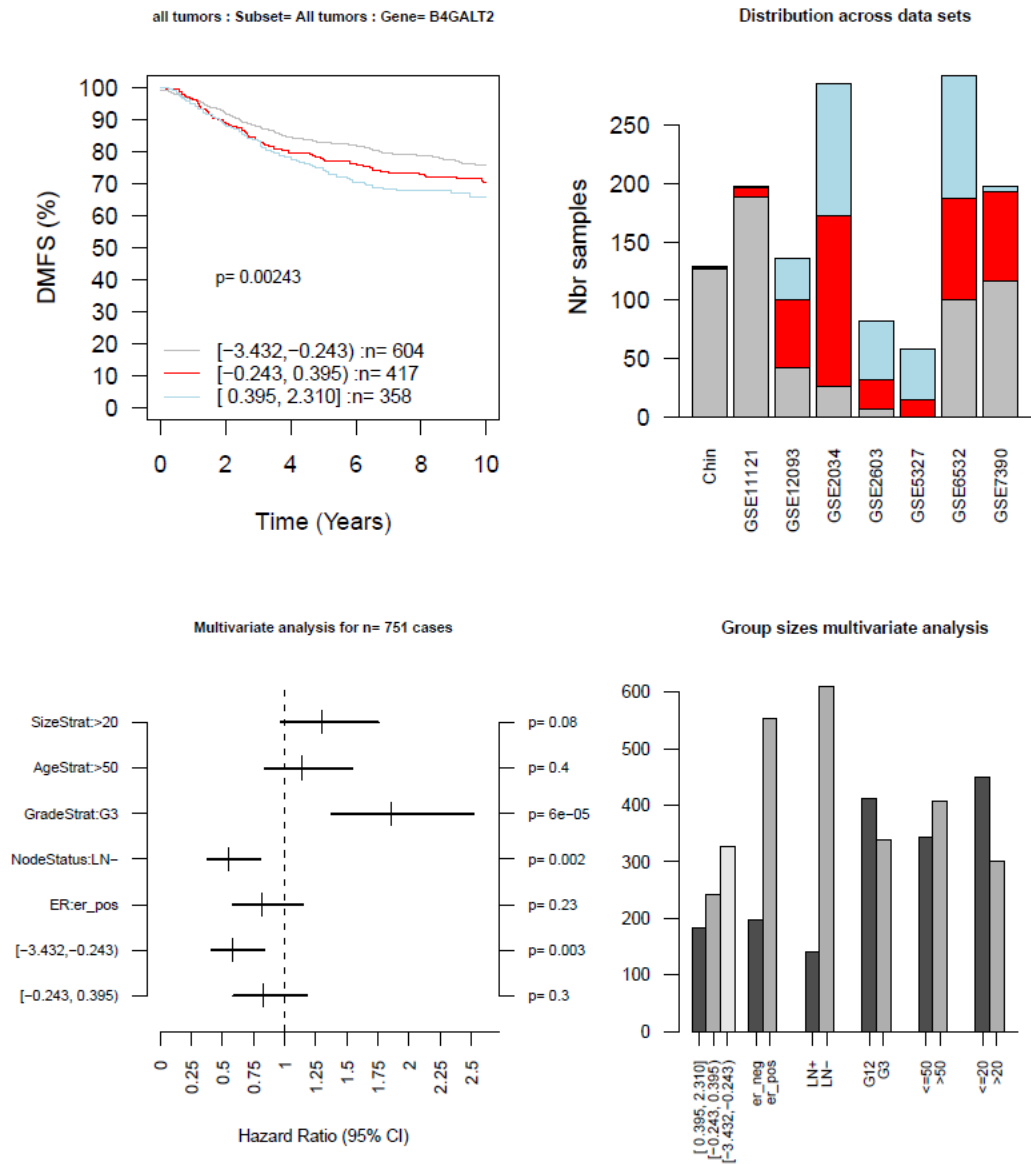


Fig 5.9: multivariate expression analysis of B4GALT2

5.1.2.4 Validation of B4GALT1 expression in cancer by Boxplot analysis of GEPIA

The gene expression of the B4GALT2 was also analyzed by Boxplot analysis of TCGA datasets in GEPIA. It was observed that the expression of B4GALT2 was upregulated in tumors, compared to the regulation in the pair normals. The results of the gene expression analysis of B4GALT2 by using the TCGA dataset has shown considerably different levels of regulation. Since the gene expression was varied, the protein expression was evaluated.

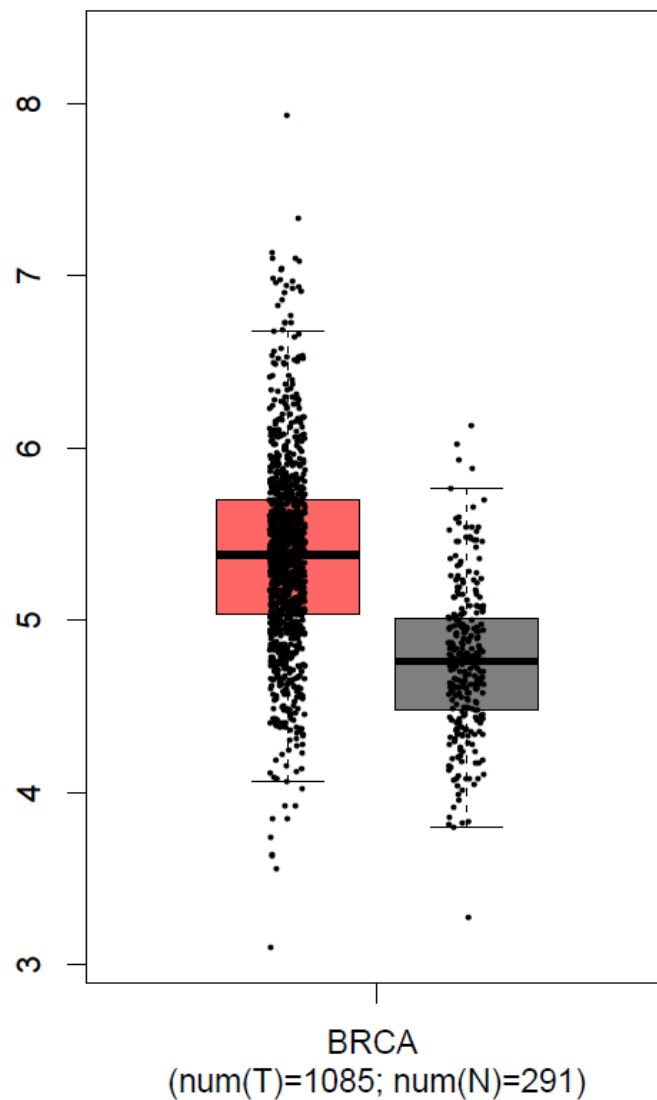
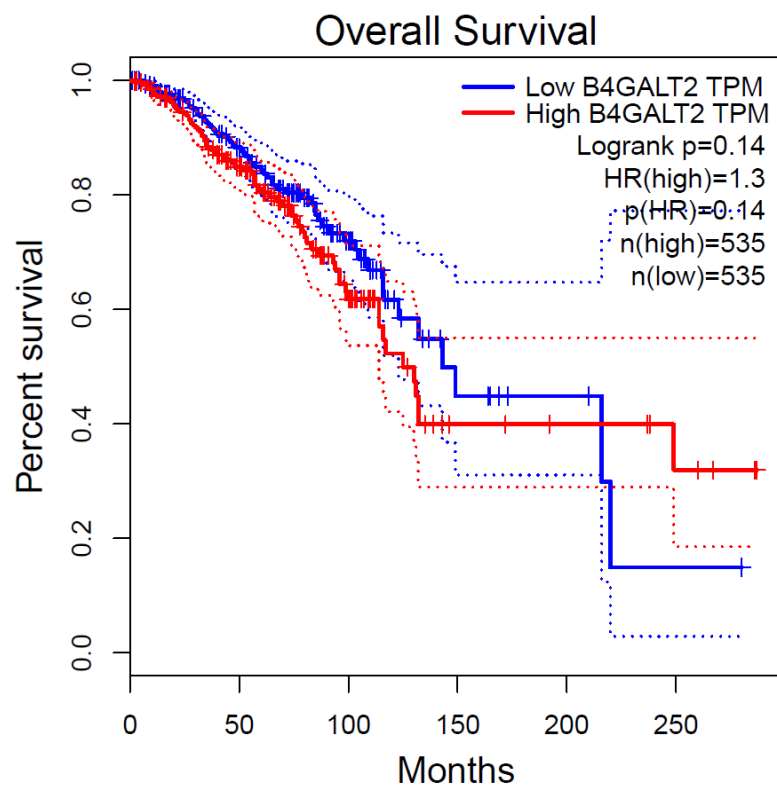


Fig 5.10 B4GALT2 expression validation by boxplot

5.1.2.5 B4GALT2 survival analysis in breast cancer:

Survival analysis using GEPIA datasets was performed for the gene B4GALT2. It was inferred from the Hazard Ratio (HR) = 0.14, that there is no significant effect between the expression levels of B4GALT1 and the survival rate of patients.



5.11 Survival analysis for Breast cancer

5.2 RNA Quantification:

The RNA concentration of the measured samples consistently showed higher values for the tumors and comparatively lower values in pair-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.

Table 5.1 RNA Quantification

No	RNA Quantification (T)	RNA Quantification (PN)	A260/A280 (T)	A260/A280 (PN)
1	284.8	578.5	2.02	2.49
2	551.8	15.6	2.04	2.14
3	613.5	36.8	2.02	2.12
4	57.6	7	1.94	2.04
5	214.3	20.1	2.11	2.15
6	167.6	21.5	2.15	2.05
7	155.7	52.9	2	2.43
8	1252.1	72.9	2.09	2.4
9	279.6	65	1.99	1.67
10	181.7	43.3	1.7	2.38
11	1610.4	30.4	2.1	2.45
12	172	13.1	2.12	2.23
13	225.1	81.1	2.12	2.21
14	319.2	69.9	2.09	2.05
15	1861	91.6	1.56	1.97
16	286.1	103.7	2	2.28
17	391	228.2	2.02	2.33
18	925.6	37.6	2.05	2.15
19	67.9	225	1.82	2.38
20	2020.7	315.2	1.02	2.3
21	25	218.3	2.12	2.31
22	290.3	33.5	2.3	2.37
23	185.4	74.2	2.15	2.17
24	150.2	105.6	2.24	2.24
25	801.6	313.1	1.97	2.23
26	1665.4	42	1.7	2.12
27	1804	33.5	2	2.17
28	1210.7	24	2.13	2.22
29	1166.9	270.3	1.95	2.28

5.3 Expression analysis

5.3.1 q-PCR Analysis of B4GALT1

qRT-PCR was carried out with the gene-specific primers for the two B4GALT genes (B4GALT1) and GAPDH as the normalising control genes, by utilizing the SensiFAST™ SYBR® Lo-ROX Kit (Bioline, UK) with respect to manufacturer's protocol in the form of 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 include: (Hold Stage: 50°C for 2 min, 95°C for 2 min) and (PCR Stage: 95°C for 15 sec and 60°C for 1 min) for 40 cycles.

5.3.1.1 Expression of B4GALT1 in cell lines

The Expression of B4GALT1 was observed in the cell lines: ZR75-1 and MCF7, by utilizing q-PCR.

(i) B4GALT1 in ZR75-1

The primers for B4GALT1 and GAPDH [table 4.2] as a normalising control gene were added to the master mix, where Z75-1 and HEK293 as a calibrator, was considered as template.

Table 5.2 Gene expression analysis of B4GALT1 in ZR75-1 cell line:

Cell lines	CT value of B4GALT1		CT value of GAPDH	
ZR75-1	22.367	22.262	13.635	13.429
HEK293	22.081	22.078	17.416	16.276

Cell lines	Test experiment (TE)		Test Control (TC)		Housekeeping Experiment (HE)		Housekeeping Control (HC)	
ZR75-1	22.367	22.262	22.081	22.078	13.635	13.429	17.416	16.276

Cell lines	$\Delta CT(TE-HE)$		$\Delta CT(TC-HC)$		$\Delta\Delta CT (\Delta CT TE - \Delta CT TC)$		$2\Delta\Delta CT$ (fold change)	
ZR75-1	7.150	8.627	2.822	4.662	4.328	3.965	0.0497	0.0640

Cell lines	Average of fold change		Standard deviation of fold change	
ZR75-1	0.0569		0.0100	

(ii) B4GALT1 in MCF-7

The primers for B4GALT1 and GAPDH [table 4.2] as a normalising control gene were added to the master mix, where MCF-7 and HEK293 as a calibrator, was considered as template.

Table 5.3 Gene expression analysis of B4GALT1 in MCF-7 cell line:

Cell lines	CT value of B4GALT1		CT value of GAPDH	
MCF-7	22.372	21.992	15.241	13.284
HEK293	22.081	22.078	17.416	16.276

Cell lines	Test experiment (TE)		Test Control (TC)		Housekeeping Experiment (HE)		Housekeeping Control (HC)	
MCF-7	22.372	21.992	22.081	22.078	13.635	13.429	17.416	16.276

Cell lines	$\Delta CT(TE-HE)$		$\Delta CT(TC-HC)$		$\Delta\Delta CT (\Delta CT TE - \Delta CT TC)$		$2\Delta\Delta CT$ (fold change)	
MCF-7	7.131	8.708	2.822	4.662	4.309	4.046	0.0504	0.0605

Cell lines	Average of fold change		Standard deviation of fold change	
MCF-7	0.0555		0.0071	

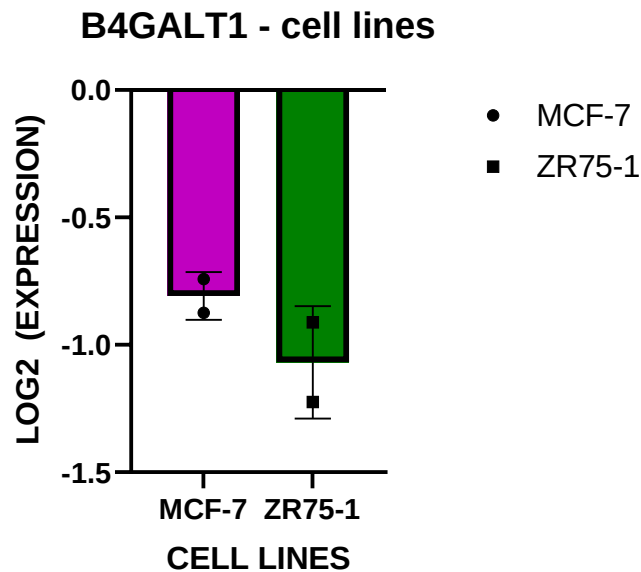


Fig 5.12 Graphical representation of the fold change expression of B4GALT1 gene in cell lines in logarithmic scale.

On comparison of the fold change expression in logarithmic scale of B4GALT1 in the cell lines, it was observed that the trend followed in the expression was like the expression Insilco analysis that was performed using GOBO [Fig 5.1]

5.3.1.2 Expression of B4GALT1 in cancerous tissue along with its corresponding paired normal:

The primers for B4GALT1 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, as a calibrator, were considered as templates and qPCR analysis was done.

Table 5.4 Cycle threshold of B4GALT1 and GAPDH - tumors

S.No	CT value of B4GALT1 of tumors		CT value of GAPDH	
1.	24.291	24.423	18.835	18.804
2.	32.810	32.149	26.993	24.260
3.	24.263	24.351	17.803	18.940
4.	26.194	26.991	19.155	19.118
5.	22.763	22.554	18.446	17.795
6.	22.980	22.282	20.275	20.201
7.	23.218	23.382	22.049	22.034
8.	21.172	20.943	16.762	17.736
9.	28.980	28.916	24.026	24.043
10.	21.515	21.844	17.318	17.711
11.	31.287	31.359	20.864	20.414
12.	22.920	22.999	18.765	18.629
13.	28.004	28.509	18.634	18.499
14.	30.542	31.181	27.575	27.759
15.	27.397	27.397	21.556	21.710
16.	28.581	29.294	21.085	21.493
17.	33.070	32.411	28.936	26.290
18	29.918	30.045	23.284	26.633
19	31.706	31.490	29.161	28.986
20	36.451	36.050	30.083	30.078
21	22.768	22.413	18.570	19.372
22	30.016	30.205	20.765	21.645

Table 5.5 Cycle threshold of B4GALT1 and GAPDH – pair normal

S.No	CT value of B4GALT1 of pair normals		CT value of GAPDH	
1.	27.040	27.083	23.979	23.921
2.	33.303	30.806	27.673	26.307
3.	27.008	26.992	23.083	23.324
4.	25.767	25.642	22.476	22.164
5.	24.790	25.054	21.227	21.367
6.	27.626	28.144	23.933	24.773
7.	24.573	24.446	22.619	22.862
8.	34.949	39.378	29.243	29.668
9.	28.981	28.699	24.714	24.405
10.	33.373	34.487	26.920	27.554
11.	27.445	26.342	24.185	24.084
12.	25.169	24.688	21.773	21.235
13.	29.265	28.643	22.069	22.515
14.	32.372	31.694	29.435	29.430
15.	31.138	31.795	28.417	29.385
16.	33.578	34.332	27.128	27.340
17.	32.924	31.181	27.175	25.919
18	31.730	30.958	29.322	29.003
19	30.860	30.961	30.457	28.441
20	33.879	34.319	27.778	27.914
21	24.942	26.282	20.141	19.725
22	34.253	34.120	24.424	24.425

Table 5.6 Cycle threshold of B4GALT1 – tumors and pair normal

S.No	Test experiment (TE)		Test control (TC)		Housekeeping experiment (HE)		Housekeeping control (HC)	
1.	24.291	24.423	27.040	27.083	18.835	18.804	23.979	23.921
2.	32.810	32.149	33.303	30.806	26.993	24.260	27.673	26.307
3.	24.263	24.351	27.008	26.992	17.803	18.940	23.083	23.324
4.	26.194	26.991	25.767	25.642	19.155	19.118	22.476	22.164
5.	22.763	22.554	24.790	25.054	18.446	17.795	21.227	21.367
6.	22.980	22.282	27.626	28.144	20.275	20.201	23.933	24.773
7.	23.218	23.382	24.573	24.446	22.049	22.034	22.619	22.862
8.	21.172	20.943	34.949	39.378	16.762	17.736	29.243	29.668
9.	28.980	28.916	28.981	28.699	24.026	24.043	24.714	24.405
10.	21.515	21.844	33.373	34.487	17.318	17.711	26.920	27.554
11.	31.287	31.359	27.445	26.342	20.864	20.414	24.185	24.084
12.	22.920	22.999	25.169	24.688	18.765	18.629	21.773	21.235
13.	28.004	28.509	29.265	28.643	18.634	18.499	22.069	22.515
14.	30.542	31.181	32.372	31.694	27.575	27.759	29.435	29.430
15.	27.397	27.397	31.138	31.795	21.556	21.710	28.417	29.385
16.	28.581	29.294	33.578	34.332	21.085	21.493	27.128	27.340
17.	33.070	32.411	32.924	31.181	28.936	26.290	27.175	25.919
18.	29.918	30.045	31.730	30.958	23.284	26.633	29.322	29.003
19.	31.706	31.490	30.860	30.961	29.161	28.986	30.457	28.441
20.	36.451	36.050	33.879	34.319	30.083	30.078	27.778	27.914
21.	22.768	22.413	24.942	26.282	18.570	19.372	20.141	19.725
22.	30.016	30.205	34.253	34.120	20.765	21.645	24.424	24.425

Table 5.7-Fold change of B4GALT1 from tumors and pair normal

S.No	ΔCT (TE-HE)		ΔCT (TC-HC)		$\Delta\Delta CT$ (ΔCT TE – ΔCT TC)		$2\Delta\Delta CT$ (fold change)	
1.	5.457	5.619	3.061	3.104	2.395	2.515	0.19006423	0.174904829
2.	5.817	7.889	5.630	4.499	0.187	3.390	0.878635884	0.095407006
3.	6.460	5.411	3.925	3.668	2.535	1.743	0.172541284	0.29867513
4.	7.039	7.873	3.291	3.478	3.748	4.395	0.074417575	0.047532519
5.	4.317	4.760	3.563	3.826	0.754	0.933	0.592890738	0.523614155
6.	2.704	2.082	3.693	3.371	-0.988	-1.289	1.983786069	2.443979619
7.	1.170	1.348	1.954	1.584	-0.784	-0.236	1.722392916	1.177355436
8.	4.409	3.207	5.706	9.710	-1.297	-6.503	2.456636265	90.69269392
9.	4.955	4.873	4.267	4.294	0.688	0.579	0.620824659	0.669585848
10.	4.197	4.132	6.453	6.933	-2.256	-2.801	4.777740785	6.967826837
11.	10.423	10.945	3.260	2.258	7.163	8.688	0.006980229	0.002425404
12.	4.155	4.370	3.396	3.454	0.759	0.916	0.590895299	0.52988728
13.	9.371	10.010	7.196	6.128	2.174	3.882	0.221572315	0.067840648
14.	2.968	3.422	2.938	2.264	0.030	1.158	0.979516951	0.448102249
15.	5.842	5.688	2.722	2.411	3.120	3.277	0.115035099	0.103153462
16.	7.495	7.801	6.449	6.992	1.046	0.809	0.484286844	0.570637007
17.	4.134	6.121	5.749	5.262	-1.615	0.859	3.064075429	0.551278016
18.	6.634	3.412	2.408	1.955	4.226	1.457	0.053439409	0.36427897
19.	2.545	2.504	0.402	2.520	2.142	-0.017	0.226492678	1.011512273
20.	6.368	5.972	6.101	6.405	0.267	-0.432	0.83101059	1.349431801
21.	4.198	3.041	4.801	6.557	-0.603	-3.516	1.518623385	11.44011269
22.	9.250	8.560	9.829	9.696	-0.579	-1.136	1.493369653	2.197644026

Table 5.8 Average fold change and standard deviation – B4GALT1

S.No	Standard deviation	Average
1.	0.010719315	0.182484529
2.	0.55382645	0.487021445
3.	0.089190098	0.235608207
4.	0.019010605	0.060975047
5.	0.048985942	0.558252447
6.	0.325405979	2.213882844
7.	0.385399698	1.449874176
8.	62.39231471	46.57466509
9.	0.034479368	0.645205254
10.	1.548624699	5.872783811
11.	0.003220748	0.004702817
12.	0.043139184	0.560391289
13.	0.108704704	0.144706481
14.	0.37576694	0.7138096
15.	0.008401586	0.109094281
16.	0.061058786	0.527461926
17.	1.776816091	1.807676722
18	0.219796762	0.208859189
19	0.555092679	0.619002475
20	0.366579154	1.090221195
21	7.015552365	6.479368036
22	0.497997185	1.84550684

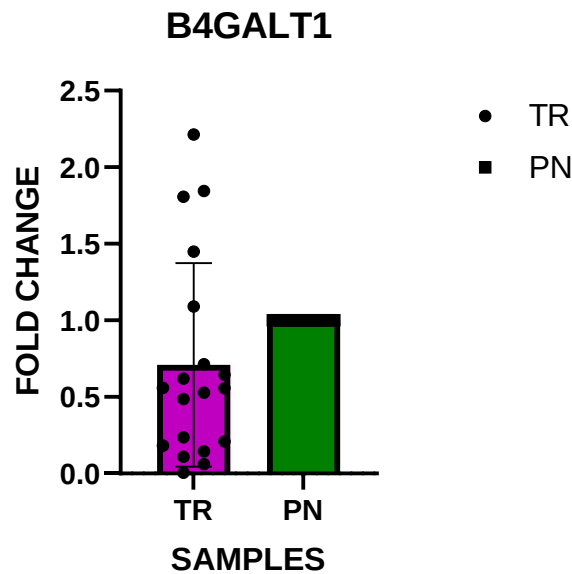


Fig 5.13 Graphical representation of the fold change expression of B4GALT1 gene in tumors and their corresponding paired normal.

On comparison of the fold change expression of B4GALT1 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 B4GALT1 was found to similar to that of the expression of the same in CPTAC samples. [Fig 5.4]

Calculation of ΔCT value

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

To calculate the ΔCT value of B4GALT1, $\Delta CT = \text{ct value of targeted gene} - \text{ct value of control gene}$.

For example, the subtraction of the CT value of GAPDH from the target gene CT value was found to be 10.101

$$\Delta CT = 26.856 - 16.755 = 10.101$$

Calculation the $\Delta\Delta CT$ value

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

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

For example, the value of the $\Delta\Delta CT$ Targeted B4GALT1 gene is 5.764.

which is calculated by,

$$\Delta\Delta CT = 10.101 - 4.337 = 5.764$$

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 B4GALT1 is calculated from $\Delta\Delta ct$ value is 0.0184019.

$$\text{Fold change} = 2^{-(5.764)} = 0.0184019$$

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

5.3.1.3 High Expression in Tumor

Few samples were found to be highly expressed in tumor than in paired normal. These samples indicate that expression of B4GALT1 is high in them compared to that of other samples.

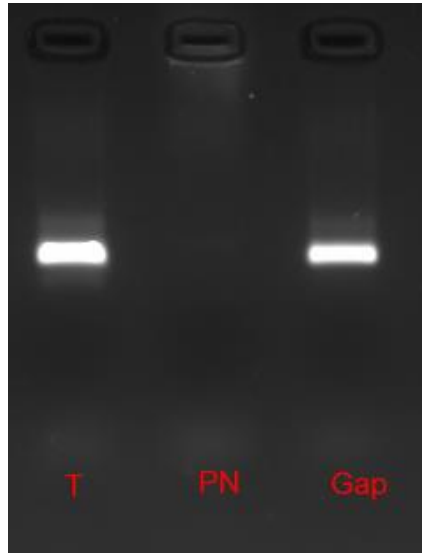


Fig 5.14 High expression of B4GALT1 in tumor and low expression of B4GALT1 in paired normal

5.3.1.4 Expression in Paired Normal Compared to Corresponding Tumors

In some samples, expression of B4GALT1 was found to be low than that of the corresponding paired normal.

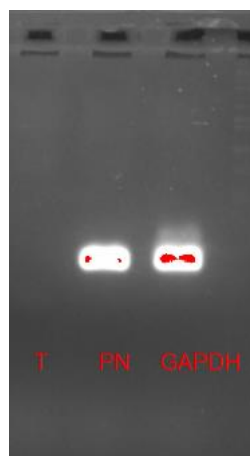


Fig 5.15 B4GALT1 expressed in Paired Normal compared to expression in corresponding tumor.

5.3.1.5 Low Or No Expression Of B4GALT1 In both cancererous Tissues and Corresponding Paired Normal

In few samples, expression of B4GALT1 was found to be low in both, cancerous tissue and in their corresponding paired normal.



Fig 5.16 No expression of B4GALT1 in both tumors and their corresponding paired normal.

5.3.2 q-PCR Analysis of B4GALT2

qRT-PCR was carried out with the gene-specific primers for the B4GALT gene (B4GALT2) and GAPDH as the normalising control genes, by utilizing the SensiFAST™ SYBR® Lo-ROX Kit (Bioline, UK) with respect to manufacturer's protocol in the form of 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 include: (Hold Stage: 50°C for 2 min, 95°C for 2 min) and (PCR Stage: 95°C for 15 sec and 60°C for 1 min) for 40 cycles.

5.3.2.1 Expression of B4GALT2 in cell lines

The Expression of B4GALT2 was observed in the cell lines: ZR75-1 and MCF7, by utilizing q-PCR.

(i) B4GALT2 in ZR75-1

The primers for B4GALT2 and GAPDH [table 4.2] as a normalising control gene were added to the master mix, where Z75-1 and HEK293 as a calibrator, was considered as template.

Table 5.9 Gene expression of B4GALT2 in ZR75-1 cell line:

Cell lines	CT value of B4GALT2		CT value of GAPDH	
ZR75-1	25.563	25.081	13.635	13.429
HEK293	24.694	22.947	17.416	16.276

Cell lines	Test experiment (TE)		Test Control (TC)		Housekeeping Experiment (HE)		Housekeeping Control (HC)	
ZR75-1	25.563	25.081	24.694	22.947	13.635	13.429	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)	
ZR75-1	11.928	11.447	7.277	6.671	4.169	3.964	0.0555	0.0640

Cell lines	Average of fold change	Standard deviation of fold change
ZR75-1	0.1985	0.2402

(ii) B4GALT2 in MCF-7:

The primers for B4GALT2 and GAPDH [table 4.2] as a normalising control gene were added to the master mix, where MCF-7 and HEK293 as a calibrator, was considered as template.

Table 5.10 Gene expression of B4GALT2 in MCF-7 cell line:

Cell lines	CT value of B4GALT2		CT value of GAPDH	
MCF-7	23.800	25.503	15.241	13.284
HEK293	24.694	22.947	17.416	16.276

Cell lines	Test experiment (TE)		Test Control (TC)		Housekeeping Experiment (HE)		Housekeeping Control (HC)	
MCF-7	23.800	25.503	24.694	22.947	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	8.559	12.218	9.274	7.277	-0.715	4.941	1.6419	0.0325

Cell lines	Average of fold change	Standard deviation of fold change
MCF-7	0.837227	1.137976

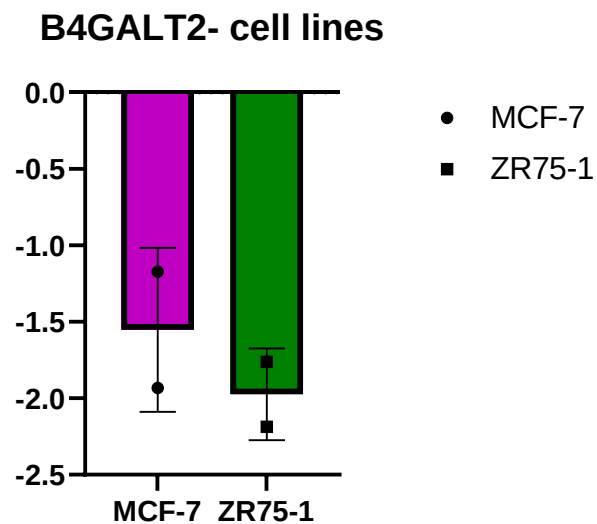


Fig 5.17 Graphical representation of the fold change expression of B4GALT2 gene in cell lines in logarithmic scale.

On comparison of the fold change expression in logarithmic scale of B4GALT2 in the cell lines, it was observed that the trend followed in the expression was like the expression Insilco analysis that was performed using GOBO [Fig 5.7]

5.3.2.2 Expression of B4GALT2 in cancerous tissue along with its corresponding paired normal

The primers for B4GALT2 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, as a calibrator, were considered as templates and qPCR analysis was done.

Table 5.11 Cycle threshold of B4GALT2 and GAPDH - tumors

S.No	CT value of B4GALT2 of tumors		CT value of GAPDH	
1	32.772	32.133	24.461	24.526
2	27.893	28.261	17.803	18.940
3	32.711	32.812	19.155	19.118
4	26.870	26.919	20.275	20.201
5	29.572	29.399	22.049	22.034
6	31.978	31.535	24.026	24.043
7	22.963	23.478	17.318	17.711
8	29.175	29.487	20.864	20.414
9	29.725	29.900	18.634	18.499
10	31.249	31.107	21.556	21.710
11	31.460	31.458	21.085	21.493
12	35.699	34.976	28.936	26.290
13	32.402	31.940	26.408	22.829
14	31.869	31.957	23.284	26.633

15	26.010	26.755	18.570	19.372
16	27.573	27.482	18.913	18.996
17	34.825	36.126	28.483	27.424
18	32.266	33.132	20.765	21.645

Table 5.12 Cycle threshold of B4GALT2 and GAPDH – pair normal

S.No	CT value of B4GALT2 of pair normal		CT value of GAPDH	
1	40	40	24.037	24.107
2	28.832	29.198	23.083	23.324
3	28.038	28.428	22.476	22.164
4	31.453	31.025	23.933	24.773
5	28.502	28.969	22.619	22.862
6	28.595	29.267	24.714	24.405
7	33.759	32.819	26.920	27.554
8	29.062	29.460	24.185	24.084
9	31.060	31.983	22.069	22.515
10	33.234	33.685	28.417	29.385
11	35.175	36.477	27.128	27.340
12	36.197	33.653	27.175	25.919
13	35.963	33.518	27.539	27.539
14	37.361	36.126	29.322	29.003
15	27.933	28.200	20.141	19.725
16	30.299	30.936	24.743	24.646
17	34.927	35.150	29.020	27.927
18	35.544	35.187	24.424	24.425

Table 5.13 Cycle threshold of B4GALT2 – tumours and pair normal

S.No	Test experiment (TE)		Test control (TC)		Housekeeping experiment (HE)		Housekeeping control (HC)	
1.	32.772	32.133	40	40	24.461	24.526	24.037	24.107
2.	27.893	28.261	28.832	29.198	17.803	18.940	23.083	23.324
3.	32.711	32.812	28.038	28.428	19.155	19.118	22.476	22.164
4.	26.870	26.919	31.453	31.025	20.275	20.201	23.933	24.773
5.	29.572	29.399	28.502	28.969	22.049	22.034	22.619	22.862
6.	31.978	31.535	28.595	29.267	24.026	24.043	24.714	24.405
7.	22.963	23.478	33.759	32.819	17.318	17.711	26.920	27.554
8.	29.175	29.487	29.062	29.460	20.864	20.414	24.185	24.084
9.	29.725	29.900	31.060	31.983	18.634	18.499	22.069	22.515
10.	31.249	31.107	33.234	33.685	21.556	21.710	28.417	29.385
11.	31.460	31.458	35.175	36.477	21.085	21.493	27.128	27.340
12.	35.699	34.976	36.197	33.653	28.936	26.290	27.175	25.919
13.	32.402	31.940	35.963	33.518	26.408	22.829	27.539	27.539
14.	31.869	31.957	37.361	36.126	23.284	26.633	29.322	29.003
15.	26.010	26.755	27.933	28.200	18.570	19.372	20.141	19.725
16.	27.573	27.482	30.299	30.936	18.913	18.996	24.743	24.646
17.	34.825	36.126	34.927	35.150	28.483	27.424	29.020	27.927
18.	32.266	33.132	35.544	35.187	20.765	21.645	24.424	24.425

Table 5.14-Fold change of B4GALT2 from tumors and pair normal

S.No	ΔCT (TE- HE)		ΔCT (TC- HC)		$\Delta\Delta CT$ (ΔCT TE – ΔCT TC)		$2\Delta\Delta CT$ (fold change)	
1.	8.310	7.607	15.963	15.893	-7.653	-8.286	201.2130772	312.1576269
2.	10.091	9.321	5.749	5.874	4.342	3.447	0.049323489	0.091665257
3.	13.556	13.694	5.562	6.264	7.994	7.429	0.003922111	0.005801741
4.	6.594	6.718	7.519	6.253	-0.925	0.465	1.898688258	0.724427901
5.	7.523	7.365	5.883	6.107	1.640	1.258	0.320852368	0.418066987
6.	7.952	7.492	3.880	4.862	4.072	2.630	0.059469321	0.161549537
7.	5.646	5.767	6.839	5.265	-1.194	0.502	2.287571979	0.706161349
8.	8.311	9.073	4.877	5.376	3.434	3.697	0.092495376	0.077104429
9.	11.091	11.401	8.991	9.469	2.100	1.933	0.233241226	0.261959418
10.	9.693	9.398	4.817	4.301	4.876	5.097	0.034062646	0.029220947
11.	10.375	9.965	8.047	9.136	2.328	0.829	0.19915646	0.562965252
12.	6.763	8.686	9.023	7.734	-2.259	0.952	4.788174425	0.516866286
13.	5.993	9.111	8.425	5.979	-2.431	3.132	5.39450974	0.114097083
14.	8.585	5.323	8.040	7.123	0.546	-1.800	0.685036252	3.48168023
15.	7.440	7.383	7.793	8.474	-0.352	-1.091	1.276717983	2.130345375
16.	8.660	8.485	5.556	6.290	3.104	2.195	0.116344955	0.218320233
17.	6.342	8.702	5.907	7.223	0.434	1.479	0.740019751	0.358837447
18.	11.500	11.487	11.120	10.762	0.380	0.725	0.768491884	0.605200081

Table 5.15 Average fold change and standard deviation – B4GALT2

S.No	Standard deviation	Average
1.	78.44964344	256.685352
2.	0.029940152	0.070494373
3.	0.001329099	0.004861926
4.	0.830327462	1.311558079
5.	0.068741116	0.369459678
6.	0.072181613	0.110509429
7.	1.11822618	1.496866664
8.	0.010883043	0.084799902
9.	0.020306829	0.247600322
10.	0.003423598	0.031641797
11.	0.257251664	0.381060856
12.	3.020270949	2.652520355
13.	3.733815597	2.754303412
14.	1.977525921	2.083358241
15.	0.603605717	1.703531679
16.	0.072107411	0.167332594
17.	0.269536592	0.549428599
18	0.115464741	0.686845982

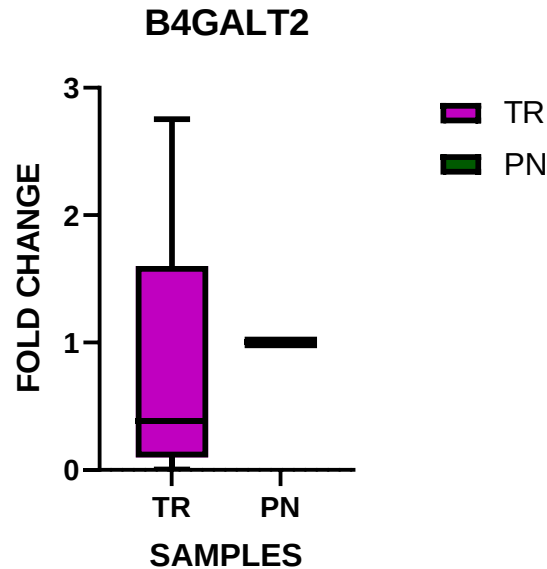


Fig 5.18 Graphical representation of the fold change expression of B4GALT2 gene in tumors and their corresponding paired normal.

On comparison of the fold change expression of B4GALT2 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 B4GALT2 was found to similar to that of the expression of the same in TCGA samples. [Fig 5.8]

Calculation of ΔCT value

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

To calculate the ΔCT value of B4GALT2, $\Delta CT = \text{ct value of targeted gene} - \text{ct value of control gene}$.

For example, the subtraction of the CT value of GAPDH from the target gene CT value was found to be 12.365.

$$\Delta CT = 36.731 - 24.366 = 12.365.$$

Calculation the $\Delta\Delta CT$ value

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

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

For example, the value of the $\Delta\Delta CT$ Targeted B4GALT2 gene is 0.943.

which is calculated by,

$$\Delta\Delta CT = 12.365 - 11.422 = 0.943$$

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 B4GALT2 is calculated from $\Delta\Delta ct$ value is 0.52015.

$$\text{Fold change} = 2^{-(0.943)} = 0.52015.$$

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

5.4 Statistical Analysis:

5.4.1 B4GALT1 Gene Expression – Statistical Analysis:

An unpaired T-test was performed on the gene expression of B4GALT1 using GraphPad Prism Version 9.3.0. between tumors and the corresponding paired normal.

Unpaired t test

P value = 0.0639

P value summary - not significant

Significantly different ($P < 0.05$)? No

t=1.912, df=36

5.4.2 B4GALT2 Gene Expression – Statistical Analysis:

An unpaired T-test was performed on the gene expression of B4GALT2 using GraphPad Prism Version 9.3.0. between tumors and the corresponding paired normal

Unpaired t test

P value = 0.5600

P value summary – not significant

Significantly different ($P < 0.05$)? No

$t=0.5890$, $df=32$

CHAPTER 6

SUMMARY AND CONCLUSION

Expression analysis of B4GALT1 and B4GALT2 was performed and studied among breast cancer tissue samples and cell line, using q-RTPCR analysis. In the In-silico analysis, it was observed that B4GALT1 was downregulated in ZR751 cell line and slightly upregulated in MCF7 [Fig 5.1]. In contrast to B4GALT1, B4GALT2 showed downregulation in both the cell-lines ZR751 and MCF7 [Fig 5.7] and for tumor samples, in TCGA datasets, tumors showed upregulation of B4GALT1 and B4GALT2 on comparison with its paired normal, in a consistent manner [Fig 5.2 and Fig 5.8], whilst the CPTAC analysis showed upregulation of B4GALT1 proteins [Fig 5.4] When Real Time PCR was performed to determine the expression of B4GALT1 and B4GALT2 in cell lines, tumors and their corresponding paired normal, a similar trend of downregulation in cell lines and tissues was observed. Based on the sample size, it was observed that B4GALT1 and B4GALT2 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 B4GALT1, the p value was found to $p = 0.0639$, on the other hand, for B4GALT2, the p value was found to be $p = 0.5600$. Both genes had p value more than 0.05, and downregulation was overserved in B4GALT1 and B4GALT2, with no significant difference in the expression.

In conclusion, it was observed that B4GALT1 and B4GALT2 show consistent expressions to an extent, all with a few variations in the expressions and that a further study is advised on the same, with an increase in the sample sizes, to further enhance the expression analysis in 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 **JAYANTH A**

Registration Number	BT24S51319232
Paper Code/Name	BT : Biotechnology
Date	10th February 2024 (Saturday)
Time	9:30 AM to 12:30 PM
Session	Forenoon : S5
Photo ID Submitted	PAN Card (Number : CEDPJ2046G)

Examination Centre Code: 1319

Address of the Centre:

Rajarajeswari College of Engineering,
14, Ramohalli cross,
Mysore Road, Kumbalgodu,
Bengaluru, Karnataka, PIN : 560074, India.




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


T344D33
7F64C009CD765BA9FA18E96450969AAC

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

Your GATE 2024 Result [BT]

Name

JAYANTH A

Registration Number

BT24S51319232

Gender

Male

Parent's/Guardian's name

ANAND R

Date of Birth (YYYY-MM-DD)

2002-06-27

Examination Paper

Biotechnology (BT)



Photograph

A handwritten signature in blue ink, appearing to read 'Jayanth A'.

Signature

Marks out of 100[#]

24

Qualifying Marks^{##}

38.9

35

25.9

General OBC-NCL/EWS SC/ST/PwD

[#]Normalized marks in case of multisession papers (CE and CS).

^{##}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 the Score Card.



SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited A++ Grade by NAAC | 12B Status by UGC | Approved by AICTE

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Name of the student : Jayanth. A

Register Number : 40230053

Title of the Thesis : Expression analysis of B4GALT-I and B4GALT-II genes in breast cancer.

Internal Guide Name : Dr. Indhumathi. SM

External Guide Name : Dr. G. Gopal

PO / PSO / PEO / SDG	Justification
PO – 1	Engineering Knowledge
PO - 2	Problem Analysis
PO - 4	Investigation