CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Disease Pattern Identification Exploring Metabolic Pathways in Breast Cancer

by

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A thesis submitted in partial fulfillment for the degree of Master of Science

in the

Faculty of Health and Life Sciences
Department of Biosciences

2018

I dedicate this thesis to my mother and to my supervisor.

CAPITAL UNIVERSITY OF SCIENCE & TECHNOLOGY

ISLAMABAD

CERTIFICATE OF APPROVAL

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Acknowledgements

Words fail me when I think of expressing gratitude to ALMIGHTY ALLAH, who has bestowed me with more than I deserve. In humbleness, I give all praise to ALMIGHTY ALLAH, the most beneficent, the most merciful for blessing me with the ability to complete this work. All respect to his Holy Prophet (Peace Be upon Him) who enabled us to recognize our creator and My Beloved Mother and Father. I am unable to find words for expressing my heart feelings toward my supervisor Dr. Sahar Fazal, Associate professor, Department of Bioinformatics & Biosciences, Capital University of Science & Technology, Islamabad for her sincere encouragement, guidance, useful suggestions and trust in me, throughout my research. Her observations and comments helped me to establish the overall direction of the research and to move forward with investigation in depth. I just cannot thank her enough for her unconditional support. Most of the results described in this thesis would not have been obtained without a close collaboration with few teachers. I owe a great deal of appreciation and gratitude to Dr. Shaukat Iqbal Malik, Dr. Syeda Marriam, Dr. Erum Dilshad, Mr. Shahid Hussain, for their help in operating different instruments. A word thanks goes to all my friends and seniors especially, Uffaq Naaz , Shumaila Azam , Raja Nadeem , Omaid Ghayyur, Ahmad Zafar Baig, Noman Hassan Shah Paan /wala, for their support, coordination and time to time guidance. Profound thanks to them for creating the unforgettable memories regarding our extra co-curricular events. In the end, I gratefully acknowledge and thank my family for their praiseworthy contribution, love and moral support. I have no words that express my gratitude for my parents, their love, care, support, encouragement and prayers have always enlightened my way throughout my life. May ALLAH bless them all.

Abstract

Breast cancer cells display distinct metabolic characteristics according to different molecular phenotypes. There may be crosstalk with the Glycolysis and FASN signaling pathway in breast cancer cells that make it more complex to evaluate the efficiency of an anti-metabolic drug. On the other hand, this research deals with metabolic reprogramming in breast cancer using Boolean network strategy. We also concluded protein expression patterns for metabolic therapy targeting glycolysis and fatty acids synthesis in breast cancer. The effect of MAPK in the metabolic pathways can be the solution to find the new therapeutic target involving in FASN and glycolysis along with SPOT14, environmental stress and growth factor. The research on target metabolism in breast cancer will largely help us to understand the complicated mechanism by which an anti- metabolic drug improves the efficacy of cancer therapy or overcomes drug resistance.

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Abbreviations

FASN	Fatty acid synthesis
MAPK	Mitogen-activated protein kinase
HIF-1	Hypoxia-inducible factor 1-alpha
HER2	Human epidermal growth factor receptor 2
SFRPs	Srizzled receptor-related protein
mTORC1	Mammalian target of rapamycin complex 1
GF	Growth Factor
Hh	Hedgehog
Wnt/ β-catenin	Wingless/Integrated/β-catenin
NF-kB	Nuclear Factor-kapa B
STAT3	Signal tranducer and activator of transcription 3
AMPK	5' adenosine monophosphate-activated protein kinase
PI3K-AKT	Phosphatidylinositol-3-Kinase and Protein Kinase B
Ras-ERK	Ras-Extracellular Signal-Regulated Kinase
DKKs	Dickkopf proteins
FZD	Frizzled
LRP	Lipoprotein receptor-related protein
Hh-Ptch	Hedgehog-Patched
GLI-1/2/3	Zinc finger protein 1/2/3
SU (FU)	Silencer of intertwined fused
Hh PTCH1	Hedgehog pathway thwarts patched1
SMO	Relocation of smoothened
TSC1	Tuberous sclerosis complex 1
TSC2	Tuberous sclerosis complex 2

SREBP	Sterol regulatory element binding protein
ACLY	ATP citrate lyase
ACACA	Acetyl-CoA carboxylase
ACA	Acetyl-CoA
BCN	Binary Control Networks
USP2	Ubiquitin specific peptidase 2
TNBC	Triple negative breast cancers
PIK3	Phosphoinositide 3-kinase
SPEBT-1c	Transcription factor for FASN
ES	Enviromental Stress

Symbols

||	OR Operation
&&	AND Operation
!	NOT Operation
=	Is Equals to

Chapter 1

Introduction

In the modern day world, science has made great advancements for the welfare of mankind but still there are things which are continuously challenging the researchers. The advancements in technology such as Stem cell and Gene therapy etc have provided immense benefits in improving our health care systems and revolutionized the methods of disease treatment [1]. With the advent of Bioinformatics, humans are capable of storing the exponentially growing DNA, RNA, Protein sequential information from Human Genome Project and other sources in curated databases. It also provided us with framework and tools in order to analyze and interpret the massive biological information for their functional assignment which may be later used in biomedical and clinical research [2]. Despite of availability of such high throughput technologies, researchers remained unsuccessful in finding the permanent cure of certain fatal diseases.

The claims made by Genome Project at the time of its completion that they have transformed the performance of disease treatment, remained subjected to certain doubts [3]. The reason behind these doubts might be the evolution of complex diseases whose remedy is challenging for biomedical researchers. Complex diseases are not caused due to a single gene mutation (as in case of simple diseases) rather they are controlled by polygenic (Multiple genes) factors along with some environmental factors, lifestyle and are heritable in nature [4]. In such diseases, genetic factors contribute partially in disease risk and they do not exhibit apparent

inheritance patterns. This environment-gene association facilitates in imparting better insights of disease causal and later helps in development of targeted therapy [5]. Most important example of complex disease is cancer which is defined as the uncontrolled/abnormal proliferation of cells due to mutation in certain gene under the control of environmental or inherited factor. Cancer is complex in the sense that it involves a series of interaction of genetic and environmental factors that directly deregulate various mechanisms of human body such as Immune system, DNA Repair mechanism and Apoptosis etc. We know that these mechanisms consist of various signaling pathways so they in cooperation with epigenetic processes determine the phenotype of cancer [6].

Cancer is global hallmark among the diseases. It is group of diseases instead of single one. It is second major public alarming disease worldwide after cardiovascular diseases. It becomes reason for death of millions of people in every year [7]. It causes body cells to proliferate in uncontrolled manner and produce mallfunctioning in cell cycle. If it has developed in abnormal cells of the breast is called breast cancer [8]. Cancer is subdivided into two types, benign and malignant. A tumor which does not spread is called a benign tumor and which spread due to invasive properties, is called malignant. Mostly malignant tumor is more alarming than benign and their cells have ability to metastasize [9].

System Biology is a critical field for cancer studies as it provides an aggregated outlook of the modified homeostasis of signaling pathways as a result of aberrations of genome and epigenome among cancerous cells and their local environment at the level of organ/organism [10]. The network analysis provided us with the interacting model to study the individual components that linked with each other to make up complex patho-physiological pathways [11]. Genome wide association studies are being carried out in order to reveal the architecture of genome for cancerous phenotypes and development of respective therapeutics [12].

There is an extraordinary enthusiasm for seeing how the complex cell practices in living creatures rise up out of the fundamental system of molecular associations. Discrete unique models, a displaying worldview in which the dynamical factors can just take discrete states, have been progressively used to show frameworks

with countless [13]. In case of biological discrete unique models the connections among cell components, for example, proteins, mRNA, and little particles are organized such that they bolster all the confused practices cells are prepared to do, (for example, homeostasis, development, cell separation and cell division) [1]. With a specific end goal, to get a full comprehension of the connection between cell practices and their hidden system of communications, the development of educational powerful models in view of the current natural information is imperative. A few dynamical displaying systems exist, which give distinctive levels of detail in the flow, while require different measures of natural data [2][4]. Toward one side of the range, for instance, very quantitative data can be gotten from conventional differential condition models [5][8] by giving diverse response rates (e.g. interpretation/interpretation rates, affiliation/separation constants, corruption coefficients) and the biophysical/biochemical properties of the segments. At the opposite end, the subjective elements of the framework can be duplicated by a discrete unique model [9][14], which requires just the combinatorial enacting or hindering nature of the communications, and not the active subtle elements [15]. Given the amazing yet showed actuality that the basic dynamical properties of an assortment of frameworks can be recreated without knowing the estimations of the particular active parameters of the procedures included [9][14], one may think about whether there is a model-autonomous approach to surmise the dynamical properties of cell organizes just by utilizing the system topology (chart structure), that is, the personality of the segments and information about their cooperations. Verifiably, this connection amongst structure and elements was perceived from the get-go in the spearheading work of Jacob and Monod [16], Thomas [17], Kauffman [18], and Glass [19], and is a piece of the first inspiration for the investigation of discrete unique models. The regular thought is that the nearness of input circles is essential for the rise of complex dynamical properties, for example, multistability and motions. All the more particularly, by doling out a sign to the communications (+ if enacting and - if inhibitory) and to the criticism circles in the system (the indication of a circle is given by the result of the indications of its edges), the accompanying two straight forward standards were proposed by R. Thomas [20]

to relate the system structure to its flow:

1. A fundamental condition for multistability (numerous steady consistent states) is the presence of a positive input circle.

2. A vital condition for supported motions (constrain cycles) is the presence of a negative criticism circle.

Since these early works, has been done toward this path and the legitimacy of these guidelines has been exhibited both in the differential [21][22][23] [24] and discrete systems [25][26][27]. Ongoing works have even stretched out these tenets to incorporate vital as well as adequate conditions for multistability and motions [28][29].

Regardless of this advance, there is as yet a requirement for growing new instruments that relate the system structure to its progression, particularly ones that are pertinent to vast scale systems. This is an issue for a significant number of the strategies grew up until now, since a large number of them are computationally requesting and must be precisely connected to systems of little to direct size. The span of the systems is additionally an issue even in situations where scientific hypotheses are accessible, in light of the fact that as the system increments in estimate, it is likely that the conditions required in the hypotheses wind up increasingly hard to be satisfied. These impediments call for techniques that are as for the most part pertinent as could reasonably be expected.

The novel investigation strategy we show in this work has the goal of gathering the dynamical collection of a system construct absolutely in light of system topology and the combinatorial idea of the communications. Surrounded in the discrete unique system, our strategy depends on the possibility that a few gatherings of hubs in the system can just settle in a solitary or few settled states. By extending the system to unequivocally incorporate the idea of the associations (positive or negative) and the conceivably synergistic direction of each component in the system, we can distinguish these steady gatherings of hubs and utilize them to streamline the system. The outcome is an entire decrease (which specifically gives the settled purposes of the framework) or an exceptionally rearranged organizes in

which most hubs are relied upon to waver. In procedure we have clarified our strategy in more detail, including the system development and system diminishment strategies engaged with it.

Chapter 2

Literature Review

Tumor name depended on location at which it does appear like breast cancer starts in breast tissue and its known as breast cancer. It is heterogeneous disease and can be distinguished further subtypes like clinical, histological and molecular classifications systems. The clinical classification is based on the Classification of Malignant Tumors and includes stage, grade, size, affected lymph nodes and metastases. Histological, breast tumors are divided into ductal and lobular carcinomas. There are four subtypes defined by tumor marker expression: luminal sub type, Basal-like, Her2-overexpressing and normal breast-like tumors [29].

During mammary gland development different biological process occur in the mammary gland which also takes part in breast cancer development and progression [30]. In this process hormones are playing vital role. Most of cases breast carcinoma caused by misbalancing of hormones [31]. In several cases breast cancer affect woman above 50 or 50. It means that menopause and breast cancer rates are correlated with each other. Onset of menopause earlier or late, age at menarche and other reproductive factors are also having involvement of changes in hormones and metabolic processes for causing breast cancer [31].

Cellular signaling pathways are involved in planning cellular and molecular features. Alteration in these pathways either due to mutation or any other cause could be responsible for breast cancer such as Wnt/ -catenin signaling and Hedgehog pathways. Wnt/ -catenin signaling pathways play strong roles in cell development

process. In the breast tumor, it is fundamentally enacted by an autocrine signaling action of the cells [32]. Hedgehog pathway over-expression result in the basic initiation of SMO and up-control of the signaling pathway in breast cancer. Furthermore these consequences have been associated with more violent results such as triple adverse breast tumor with combined effect of Wnt and Hh [33]. Inflammation signaling pathways for example, NF-kB and signal transducer and activator of STAT 3 likewise result in breast cancer. Furthermore transcription factor like HIF-1 and HIF-2 are capable to prompt malignant tumor of the breast [34].

Re-programming of the metabolic pathways is an indication of physical modifications in development cells. The declaration of particular genes that particularly govern the rate of key metabolic pathways including glycolysis, lipogenesis, and nucleotide amalgamation are definitely transformed at different periods of tumor expansion. These progressions are generally assumed as a change of tumor cells; be that as it may, they additionally took an interest in the movement of tumor cells to attain more harsh phenotypes. It has been perceived that disease cells require a gigantic rate of metabolism to help their movement rate [35]. Malignant cells, with a specific end goal to maintain their high multiplication rates, depend not just on glycolysis, which is known as the "Warburg Effect", yet additionally on a modified lipid metabolism [36]. It is reported that aerobic glycolysis is the essential to give tumor cells with dynamism along with the building blocks for the synthesis of starches, lipids, proteins, and nucleic acids. The modified lipid break down has progressively perceived as other usual belongings of cancer cells [37]. Similar to glucose metabolism, the lipid metabolism in tumor cells is managed by the basic cancer pathways, also, is accepted to be vital for the start and development of tumors.

In spite of the fact that hormones and extracellular agents encourage metabolic correspondence between tissues and organs, enzyme isoforms and regulatory molecules support regulation of metabolic pathways for specialized cellular function. Research has enlightened role of Wnt-mediated regulation of cellular metabolism with reprogramming of tumor cell bioenergetics [38].The majority metabolic change

done by cancer cells are prolonged glucose uptake and glycolysis control of metabolic pathways in the cancer of breast has uncovered differentially directed metabolic pathways in breast malignancy. This support the acceptance of multiplication by inositol signal transduction (inositol phosphate digestion) and steroid hormones. Nutrient signaling pathways regulate Wnt signaling by activating AMPK protein. After that it inhibit anabolic metabolism with mean while altering catabolic pathways like fatty acid oxidation and glycolysis [39]. FASN metabolism and expression is triggered by many growth factor and hormone receptors activation signals. It has been found that FASN expression could be regulated by other factor instead of SPEBT-1c like p53 and lipogenesis-related protein in breast cancer [13].

Cellular system complexity of biological processes could be deeply studied by mathematical modeling. Computational models widely used to study networks and their pathways of biological system. Qualitative data of interactions between biological processes provides qualitative approaches of deep insights about cell regulatory pathway. Boolean network modeling is extremely useful in making qualitative analysis. These networks models could be use for understanding and modeling of regulatory or signaling networks [40]. Boolean systems have been effectively connected in modeling the genes and the signaling systems in an assortment of organic frameworks. They are utilized in contrary engineering of regulatory networks through which network structure could be understood [41]. Fuma et al., in 2013 analysed that Boolean networks models are useful for cancer pathways therapy and the strong finding of monotherapies were additive in their effects [42].

Modification of the lipid metabolic rate has been progressively perceived in tumor cells. Cells reprogram themselves to meet their anomalous multiplication and survival. Similar to the glucose digestion, lipid digestion in tumor cells is excessively controlled by the general oncogenic signaling pathways, in addition, is acknowledged to be basis for the beginning and movement of tumors. Because of constrained glucose, unsaturated fat can likewise be devoured through -oxidation to give key substituted vitality to tumor cell survival [43]. It is discovered that incitement of unsaturated fat oxidation may add on maintaining cell survival furthermore glucose withdrawal-initiated demise in Akt-overexpression glioblastoma.

These cells seem, to a great degree dependent to all over again lipogenesis for their advancement and survival [44]. FASN lipogenesis and glycolysis both roles were studied, in which it was analyzed that over expression of fatty acid and mall function of glycolysis pathways involved in breast cancer [13]. This research is focused to model these de novo fatty acid synthesis pathway and werberg effect on breast cancer with FASN gene inhibition as drug.

Breast cancer among different cancers is most alarming disease for both developed and developing countries. Every cancer type has unique characteristics but have universal design of development. It involves genetic and epigenetic alterations among the cancer cells. The development of cancer is associated with ambiguous interactions among tumor cells and adjacent cells [45]. Breast malignancy is the utmost common tumor and second majority reason for disease-related deaths among females; because they have a particular energy metabolic arrangement in contrast to males like estrogen and other hormones pattern [46]. According to the Cancer statistics for African Americans 2016 report, cancer is the increasing global problem and in United States the most commonly detected cancer is breast cancer in women, approximately 29% [47]. In 2008 [48] Boyle et al, found that it is the second common cancer in the world. It is accountable for 1.4 million new cases per annum. It is the most frequently detected cancer and accountable for 23% (1.38 million) of the overall new malignancy cases and 14%(458, 400) of the overall tumor demises [49].

2.1 Cancer Cellular Signal Transduction

Cellular signaling pathways are accountable for managing cellular and molecular features in major signaling pathways such as Wnt/ -catenin signaling, Hedgehog signaling [50], co-activation of Wnt/-catenin signaling and Hh signaling [33], HER2 signaling [51], PI3K-AKT and Ras-ERK pathways [52].

Wnt/ -catenin signaling pathways play vital roles in cell development process. In the breast tumor it is fundamentally triggered by an autocrine signaling action of cells. Wnt signaling is exposed by various endogenous means, two of which

include the release of Wnt action inhibitors, i.e., released DKKs and SFRPs that capacity as FZD and LRP fakes, independently [53]. Its different downstream products activate a number of processes that are associated with cancer. In the breast cancer Wnt/-catenin signaling raises aerobic glycolysis through suppressing of mitochondrial respiration by dropping the transcription of cytochrome c oxidase gene [39].

The Hh signaling pathway or Hh-Ptch, is an developmentally monitored pathway of signal transmission from the cell membrane to the nucleus. This pathway is assumed critical in the normal embryonic advancement of vertebrates [54]. Hh pathway over expression results in basic stimulation of SMO and up regulation of signaling pathway in breast cancer. The three transcription factors of Hh are GLI-1/2/3, placed in the cytoplasm and a dormant state by correspondence with basic cytoplasmic proteins, KIF7 and [SU(FU)]. In the condition of idleness Hh(PTCH1), SMO, a G protein-coupled receptor, to the cell film SMO is accountable for transduction of Hh motioning inside the cell by arranging activation of GLI translation factors. The Hh protein discharged by tumor cells adds to improving the action of stoma cells [55].

Hh and Wnt/ -catenin have been associated with more hostile results such as in triple-negative breast cancer [33]. Inter communication between these two pathways has been considered important in breast cancer. Increased nuclear activity of Gli-1 and -catenin coo related with increasing tumor stage and dual activation of both is associated with recurrence and survival. Both pathways are involved in the regulation of genes critical for drug resistance [56].

HER 1,2,3,4 is family of receptors which play significant role in different cancer types and also in normal tissues. HER-2 receptor was found to be in breast cancer cell line. It amplified into Her2 protein over expression which is associated to progression of cancer [57]. HER family appears important in breast cancer with over expression as FASN, because downstream products of HER2 protein activate PI3K/Akt/mTOR signaling pathway [58].

PI3K-AKT pathway is activated by PIK3CA, AKT, and adopter protein, furthermore alteration in tumor suppressors TSC1 and TSC2 hyperactive signaling by

mTORC1. Likewise, the Ras-ERK pathway is triggered by changes in Ras, or its downstream target Raf, inactivation of GTPase-enacting proteins [59]. Initiation of glucose transport and hexokinase by Akt could prompt generation of nucleotides and amino acids essential for cell development [60]. Initiation of the PI3K/Akt/mTOR pathway is normal in breast malignancy. The PI3K pathway includes a complicated system of interactions with numerous parallel inhibiting pathways, so its hindrance discharges negative feedback bringing about actuation of compensatory signaling pathways [61].

2.2 Cancer Cell Metabolism

Cell maturity requirements, composed with metabolic processes is associated with the production of macromolecules. Therefore maturity factor pathways that control both regular and cancer cells influence on metabolic pathways to prepare cells to meet the expanded requirement for the synthesis of macromolecules to create new daughter cells [62].The most widely recognized metabolic modification in malignant cells is increased glucose up-take and glycolysis. Estrogens may hoist the expression of peroxisome proliferator initiated receptor, Akt and enacted AMPK, which subsequently affect the metabolic process, including glucose utility, lipid up-take, lipogenesis and lipid oxidation [63].

Re-programming of metabolic pathways is an indication of physical modifications in developed cells. The appearance of particular genes that particularly govern the rate of key metabolic pathways including glycolysis, lipogenesis, and nucleotide amalgamation are definitely changed at diverse periods of tumor expansion. These progressions are normally assumed as a change of tumor cells; nonetheless, they likewise took an interest in the movement of tumor cells to end up resulting in harsh phenotypes. It has been perceived that cancer cells require a colossal rate of digestion to help their movement rate [35]. Warburg effect or Aerobic glycolysis is best fitted example of reprogrammed metabolic pathway [64]. Glycolysis is a physiological reaction of hypoxia to typical tissues, However Otto Warburg in the

1920s experiment that tumor parts and ascites cancerous cells consistently consume glucose and generate lactate, despite of oxygen accessibility. This opinion was perceived in much type of cancers cells [65]. Regulation in glycolytic disturbance permits glycolytic intermediates to supply auxiliary pathways to satisfy the metabolic supply of developing cells [64]. Normal cells which are stimulated by the growth factors, active PI3K and their downstream pathways AKT or mTOR trigger a strong anabolic activity in glycolytic flux [65]. Cancer cells quickly response to mutations that permit PI3K-AKT and mTOR pathway to signaling with minor dependant on external stimulation by growth factor [66].

One of the most important metabolic hallmarks of cancer cells is enhanced lipogenesis. Normally breast cells uses flowing lipids for the production of fundamental lipids but the cells of breast cancer generally produce fats by themselves. Under this scenario biosynthetic enzyme fatty FASN help in synthesis [13]. Raised level of lipogenic compounds and general lipogenesis have been accounted in wide types of cancers and hindering the lipgenic pathway through inhibitors cause tumor cell death [67].

2.3 Targeting Warburg Effect or Glycolysis

Otto Warburgs theory, that tumor cells consume glucose and also produce amount of lactate in the surrounding area from claiming encompassing oxygen. Impeded mitochondrial capacity prompted the broadly held misconception that tumor units depend on glycolysis [68]. Know it is clear that tumor cells reveal aerobic glycolysis because the oncogenes losses the function of tumor supressors and upregulate the PI3K pathway [69]. The general recruitment of the pathway (FASN) directed by triggering of signaling pathway, that is usually motivated in tumor cells (Fig 1). Prompt of growth factors activate PI3K-Akt pathway which further regulate different substances to endure metabolic changes [70]. Akt activates mTOR promotes anabolic program in elevated glycolytic flux and the fatty acid production by triggering of HIF-1 and bind to protein SREBP, correspondingly [64].

Ras-ERK transduction put forth huge amount of its impacts on metabolism through

Myc. Myc regulating glucose up-take, glycolysis, and the pentose phosphate pathway [71]. It is overexpresses in 30-50 percent of breast cancers [72].

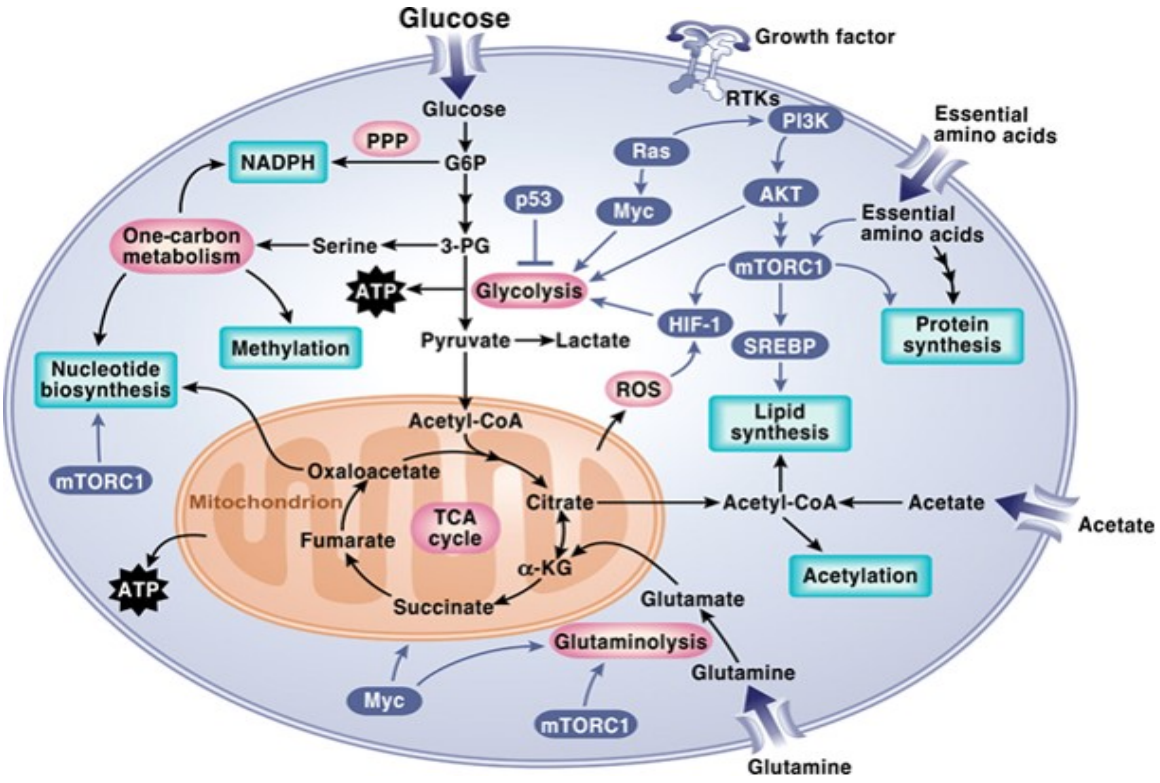

FIGURE 2.1: DeBerardinis et al., 2016.

2.4 Targeting Lipogenesis

Lipogenesis has been considered as the significant method for FA procurement in tumor cells. However, it is reported that not only lipogenes is play role in tumor progression but also exogeneous fatty acids play part in their growth [73]. Now it is broadly documented that tumors quickly display an expanded capacity to blend lipids and that this lipogenesis is firmly coupled to glucose metabolism [74]. Researches recommend that regulation of de novo FA pathway is required for carcinogenesis [75]. Different tumors show expanded endogenous FA biosynthesis regardless of extracellular lipid accessibility. Therefore, de novo FA production is suppressed in most normal cells. The elevated level of FA in cancer cells is produced by a major increment in expression and action of different enzymes involved in lipogenic pathway [13]. For example, up regulation of FASN enzyme is

correlated with breast cancer progression [76].

A factor that has to be considered as for expanded lipogenesis in tumor cells is palmitate, which is toxic to cells. Deposition of fatty acids and neutral lipids in non-adipose tissue quickly activate apoptosis. Therefore, palmitate abundance could feed back to repress endogenous FA production. A harmony between lipogenesis, lipid up-take and intracellular lipolysis would, be required to maintain lipid concentration in cell [77]. Many researches show that enzymes of liopgenesis pathway are involved in tumor progression such as ACLY, FASN and ACACA [78].

2.5 Targeting FASN Pathway

Fatty acids are very significant material for energy generation in cells for performing various reactions. It produced from both external and internal sources of body. Internal sources called de novo fatty acid synthesis by mean of enzymes production and external source through diet [79]. FASN enzyme controls metabolic activities and its uncontrolled activity produces arbitrary effects [80]. It produces long chain of fatty acids with the help of enzyme ACA and malonyl-CoA. FASN major synthesizer is palmitate fatty acid [81]. Normally cells utilizes dietary source of fatty acids for their metabolic activities but tumor cells utilizes a raised levels of de novo fatty acid synthesis. It produces significant lipids that are vital for cancer cell progression and proliferation [82]. Another problem in tumor cells is that fatty acid synthesis starts upregulate without responding to hormones in breast cancer [13].

2.6 FASN Expression in Breast Cancer

FASN enzyme is over expressed in different cancers and also in breast cancer. Endogenous de novo fatty acid synthesis high regulation in breast cancer cells has been observed. Therefore to target FASN in breast cancer through inhibitory

drugs has become attention in many researches [13]. It is over expressed in different breast cancer cell lines including hormone dependent and hormone independent [83].

2.7 FASN Structure

It is the key protein for de novo fat production and it catalyzes malonyl-CoA and acetyl-CoA to yield palmitate what's more, 16-carbon long unsaturated fats [75]. Palmitate is the first fatty acid from which other form of fatty acid can be formed. So the amount of palmitate formation can be controlled by the FASN enzyme. There are two major class of FASN. One class of FASN which is found in bacteria and plants is called type II. The other is type I which is found in humans and mammals. The major variation between type I and II are its functional domains. There are seven functional domains in both type I and type II. In type II all functional domains are independent and form multifunctional system while in type I all domains form a single bond. Any abnormalities in FASN expression affect the FASN function.

2.8 Boolean Networks and System Biology

Boolean models are based on only two asymptotic values of variable, i.e. 0 and 1 which also logically equivalence to FALSE and TRUE respectively. In biological functions these logic values are used for OFF and ON states of gene, protein or node in network. Computational models widely used to study networks and their pathways of biological system. Boolean network modeling is extremely useful in making qualitative analysis. These networks models could be used for understanding and modeling of regulatory or signaling networks [24].

Cells play out a wide assortment of undertakings amid the life expectancy of an organism like cell division, embryonic development, insusceptibility, cell expansion, tissue repair. Different correspondence mechanisms like signs of compounds exist

among and inside the cells to execute these assignments in an organized way [25]. Cells are touchy to their particular synthetic signals and react differentially to their ligands. Cell signaling directions central elements of a cell and is in charge of data stream inside the cell [84]. Signaling pathways are comprised by a gathering of atoms, cooperating to play out a particular cell capacity. Signaling procedure begins when the first particle gets a concoction motion from an additional cell signaling atom (ligand) and it actuates different particles. Any variation from the norm because of these chemical signs can cause distinctive clutters. One viable method for examining and mediation configuration is through the modeling of these pathways. Dynamic modeling of cell pathways has been continuing for over 10 years now and it has developed itself into a solid collection of writing. The immense assortment of cell pathway models found in writing contains paired models as well. Or maybe, paired models are pervasive in system arranged methodologies. Additionally, such models require far less parameterization. Up until this point, the dynamic models have been for the most part utilized for examination purposes, for example, bifurcation or attractor investigation. It is elusive such cell models for controller/intercession plan. Some portion of the reason is the absence of parameter information in such models. Paired models frequently don't experience the ill effects of this entanglement. In any case, such models can't be tended to by immense assortment of control outline hypothesis. Zanudo and Albert [85] have proposed a steady theme based mediation plan technique. This methodology is material to Boolean Networks yet the procedure isn't amiable to control hypothesis. Be that as it may, as of late D Cheng and his partners [25] have concocted framework and controller outline hypothesis particularly relating to twofold esteemed state space frameworks or BCN. There is a desperate need to apply these strategies to the twofold powerful models of cell pathway to design intercessions. Having experienced this group of work, one must yield that the strategy does not render itself effectively to controller configuration as could be found on account of traditional state space frameworks. In this work, we have planned a mediation for FASN pathway to direct it from illness attractors to sound attractors. First the incorporated system of FASN with glycolysis and lipogenesis is distinguished

from the literature. Attractor investigation is performed to separate unhealthy attractors from the normal attractors. At that point a mediation is intended to get the pathway from the unhealthy state to the normal state. The magnificence of this methodology is that broad model parameterization isn't required and the controller/intercession can likewise be connected through existing gene treatment techniques, for example, plasmid vectors or existing medications.

2.9 Problem Statement

A Boollean network approach is established to find the interaction between the two pathways targeting cellular metabolism in metabolic therapy, involving glycolysis and fatty acid synthesis in breast cancer.

2.10 Objectives

1. To find the interactive nodes between FASN signaling pathway and glycolysis. These interactive nodes can help finding new ways of treating breast cancer.

2. To find the expression pattern of these new targets using Boolean pathways.

2.11 Scope

This research might open the new ways of treating breast cancer taking system biology approach.

Chapter 3

METHODOLOGY

3.1 Generation of FASN Integrated Pathway for Attractors Analysis

Boolean Network works on two states either on (1) or off (0). It show that a gene or node can be expressed or not, or it is active or inactive. It also shows that it is below or above the threshold concentration. If it is above the threshold it will be on or (1) and if it is below the threshold it will be (0). The future value of the node or gene can be determined by the neighbor node by using the boolean logical rule. In FASN signaling pathway all nodes are logically connected and it have either 1 or 0 state. The state of any node depends on its neighbor nodes with its logical relationship. Than using the STP on logical matrices give the logical transition matrix for the whole pathway shown in figure 1 which only comprises 1 and 0.The utilization of the Boolean structure and the general nonconcurrent refreshing plan at that point maps the issue of finding the rate-invariant powerful conduct of a cell organize into finding the attractors of a Boolean system.

3.2 Boolean Model

The dynamical Boolean model of growth factor signaling pathway can be formulated by using the Boolean rule [24] [25]. These rules are based on the logical interaction of signaling pathway nodes. If a node directly regulates another node than the future value of this node depend on the past value of that node. If the node inhibited the another node than the future value of this node will be the negative of this, like if a node A is inhibited by another node B than $A(k+1) = \neg B$. If there is a node which depends on more than one node than its future value can be decided by checking the regulation or inhibition of this node. If both the node regulate than and will be used and can written as $A(k+1) = B \wedge C$.If one regulate and other inhibit than OR will be used $A(k+1) = B \vee C$.A, B and C are three nodes. Using these simple rules the dynamical Boolean model is formulated for growth factor signaling pathway which are:

$Ras = GF\&\&(!MAPK)$	$MAPK = FASN_MAPK$
$FASN_MAPK = FASN\&\&MAPK$	$PIK3 = RTKs\&\&Ras$
$MyC = Ras$	$AkT = PIK3$
$Glycolysis = (MyC\|\|AkT\|\|HIF_1)\|\|(!P53)$	$mTORC_1 = AkT$
$PSREBP1c = mTORC_1\&\&HIF_1$	$SREBPc = PSREBP1c$
$FASN = SREBPc\|\|SPOT14\|\|ES$	$FattyAcid = FASN$ $\|\|TC\|\|AcetyleCoA$
$AcetyleCoA = FattyAcid\|\|Pyruvate$	$Palmitate = AcetyleCoA\|\|$ $GF\|\|MalonylCoA$
$MalonylCoA = AcetyleCoA$	$Lactate = Glycolysis$

Above mentioned rules give the dynamical Boolean model for growth factor signaling pathway.

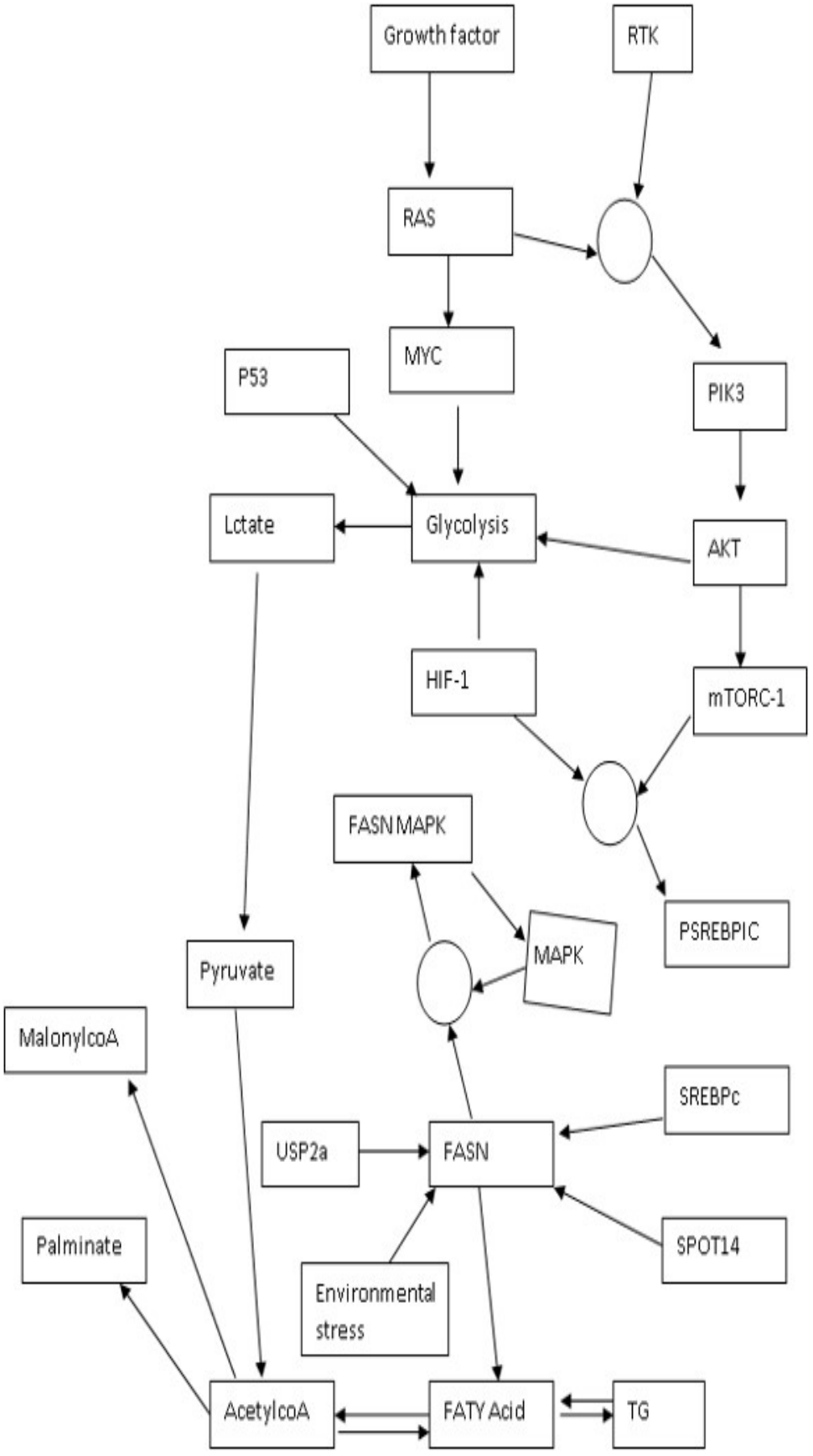

FIGURE 3.1: FASN pathway, arrow represent regulation, double arrow represent feedback regulation; circle represent composite regulation of a protein and blocks represents proteins or nodes in FASN pathway.

3.3 Finding the Attractors of a Boolean Model

The required pattern that predicts the normality and abnormality in the FASN signaling pathway are shown in the table 1by using BoolSim and the literature surveyed during the research.

Table 3.2: The attractors of FASN and lipogenesis network. This table displays the nodes states for all conceivable combinations of input signals in the presence of signal (GF ,SPOT 14, Environmental stress, USP2a)

Node	GF=ON SPOT14=OFF ES=OFF MAPK=OFF	GF=OFF SPOT14=ON ES=ON MAPK=ON	GF=ON SPOT14=OFF ES=OFF MAPK=ON	GF=ON SPOT14=ON ES=OFF MAPK=ON	GF=OFF SPOT14=ON ES=OFF MAPK=ON	GF=OFF SPOT14=ON ES=ON MAPK=OFF	GF=OFF SPOT14=OFF ES=ON MAPK=ON
RAS	OFF	OFF	ON	OFF	OFF	Oscillation	OFF
GF	ON	OFF	ON	ON	OFF	OFF	OFF
MAPK	ON	ON	OFF	ON	ON	Oscillation	ON
FASN-MAPK	ON	ON	OFF	ON	ON	OFF	ON
FASN	ON	ON	ON	ON	ON	Oscillation	ON
PIK3	OFF	OFF	ON	OFF	OFF	OFF	OFF
RIKs	ON	ON	ON	ON	ON	Oscillation	ON
MYC	OFF	OFF	ON	OFF	OFF	OFF	OFF
AKT	OFF	OFF	ON	OFF	OFF	Oscillation	OFF

Glycolysis	ON	ON	ON	ON	ON	Oscillation	ON
HIF-1	ON	ON	ON	ON	ON	Oscillation	ON
P53	ON	ON	ON	ON	ON	Oscillation	ON
Mtorc-1	OFF	OFF	ON	OFF	OFF	OFF	OFF
PSREBPC	OFF	OFF	ON	OFF	OFF	Oscillation	OFF
SREBPC	OFF	OFF	ON	OFF	OFF	OFF	OFF
SPOT14	ON	OFF	OFF	ON	ON	Oscillation	OFF
ES	ON	ON	OFF	OFF	OFF	Oscillation	ON
Fattty Acid	ON	ON	ON	ON	ON	Oscillation	ON
TC	ON	ON	ON	ON	ON	Oscillation	ON
AcetylCoA	ON	ON	ON	ON	ON	Oscillation	ON
Pyruvate	ON	ON	ON	ON	ON	Oscillation	ON
Palmitate	ON	ON	ON	ON	ON	Oscillation	ON
MalonylCoA	ON	ON	ON	ON	ON	Oscillation	ON
Lactate	ON	ON	ON	ON	ON	Oscillation	ON

The whole methodology been followed in the research can also be represented by blocked diagramed showed in the following figure 3.1. In table 3.2 we show the state of the nodes for each and every possible blend of data motions inside sight of antigen (GF, SPOT 14, Environmental Stress, and USP2a). For straightforwardness, we simply demonstrate which node influence and which of them balance out in a persevering point of interest and not the genuine attractor, which would fuse all the framework expresses that the nodes that waver can visit close by the advances between these states. The signal mixes are showing up in the table, with some other motivating force for the other data signals.

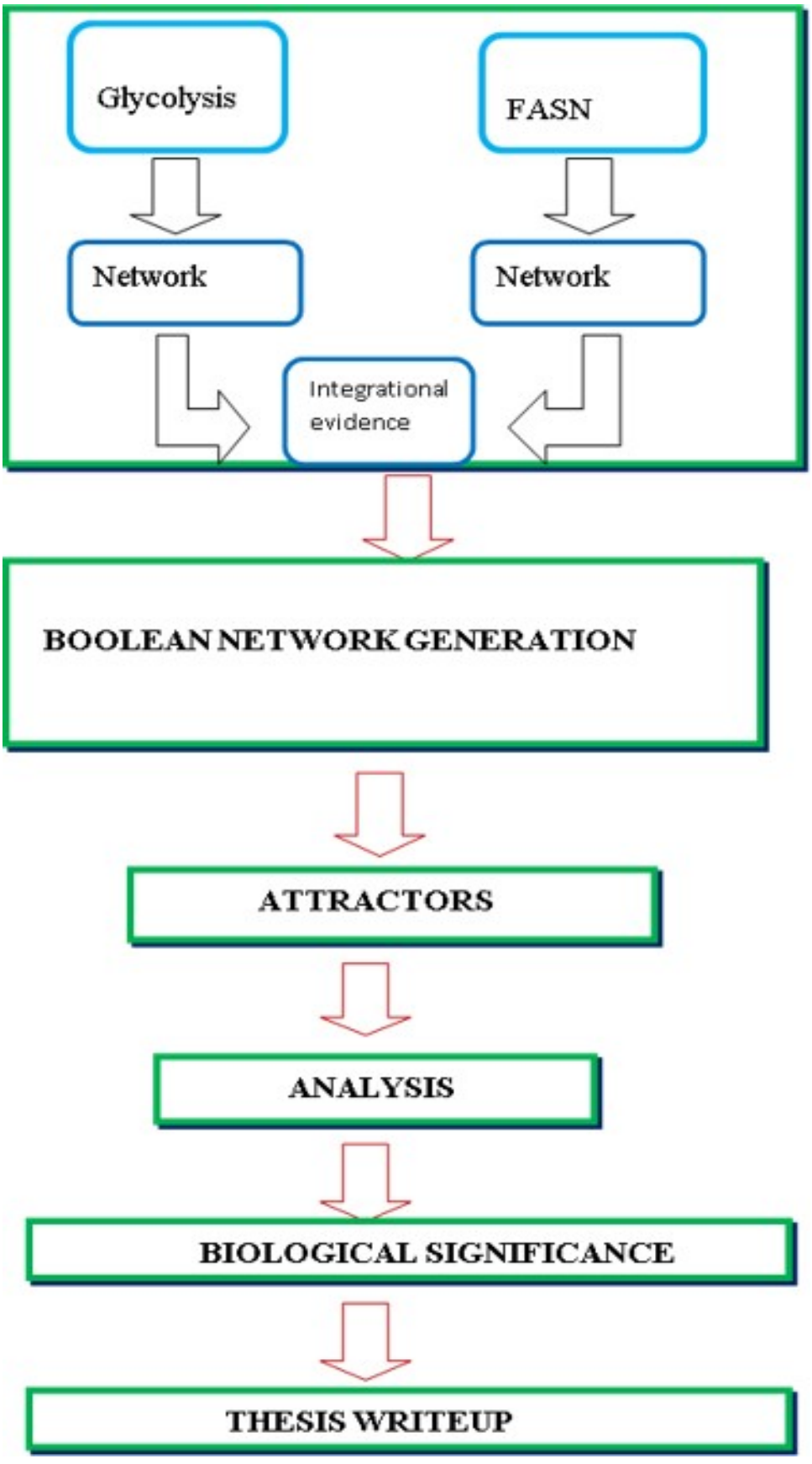

FIGURE 3.2: Block Diagram of Methodology.

Chapter 4

RESULTS AND DISCUSSION

Breast cancer currently has the most astounding rate of tumor in ladies. This is credited to the molecular grouping of breast cancer in view of the FASN, directed treatment and other adjuvant treatments that drag out the general survival and incredibly diminish the mortality of this malady. However, mortality stays high for privately progressed and metastatic malignancy. Regardless we need compelling techniques for treatment when tranquilize resistance happens and repeat and metastasis grows, particularly in TNBC.

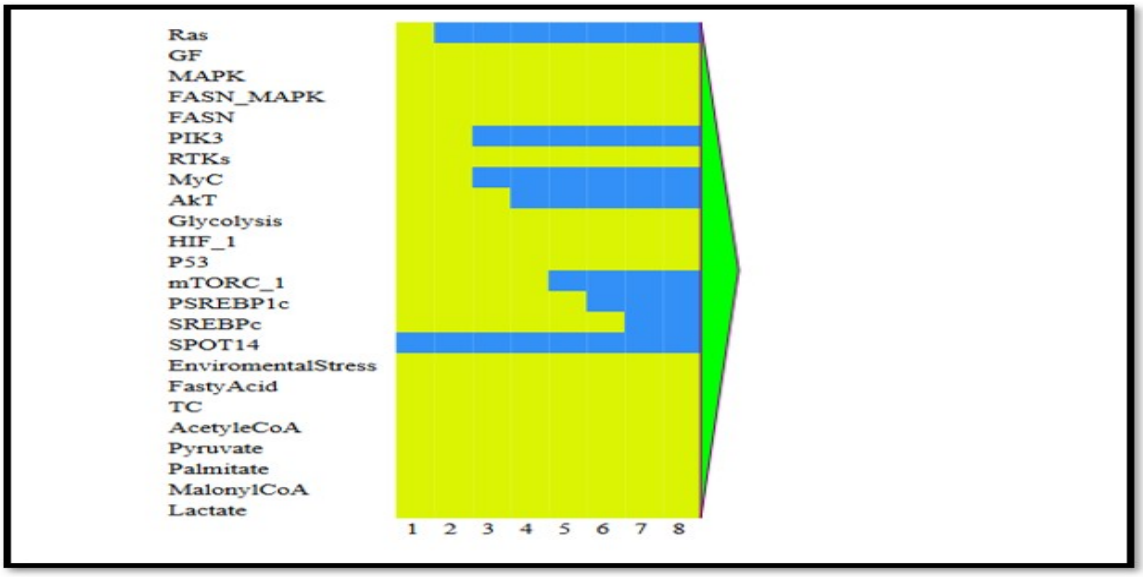

FIGURE 4.1: Protein expression pattern in glycolysis interacting FASN pathway when SPOT14 is OFF.

We applied seven conditions as mention in Table 1. After generation of network key nodes (proteins) were identified that are GF, Environmental stress, SPOT 14 were equally involved in FASN and glycolysis. In which one new node was identified as MAPK was found as an interacting node between FASN signaling pathway and glycolysis. We observed all these protein (nods) by using the seven different conditions that are mentioned in Table 1. Figure 4.1 represent the condition is GF=ON, SPOT 14=OFF, Environmental Stress=ON, MAPK=ON against which RAS, PIK3, MYC, AKT, mTORC-1, PSREBP1C, SREBPC, was OFF and GF, MAPK, FASN-MAPK, FASN, RIKs, Glycolysis, HIF-1, P53, SPOT14, Environmental stress, Fatty acid, Tc, AcetyleCoA, Pyruvate, Palmitate, MalonylCoA, Lactate was ON.

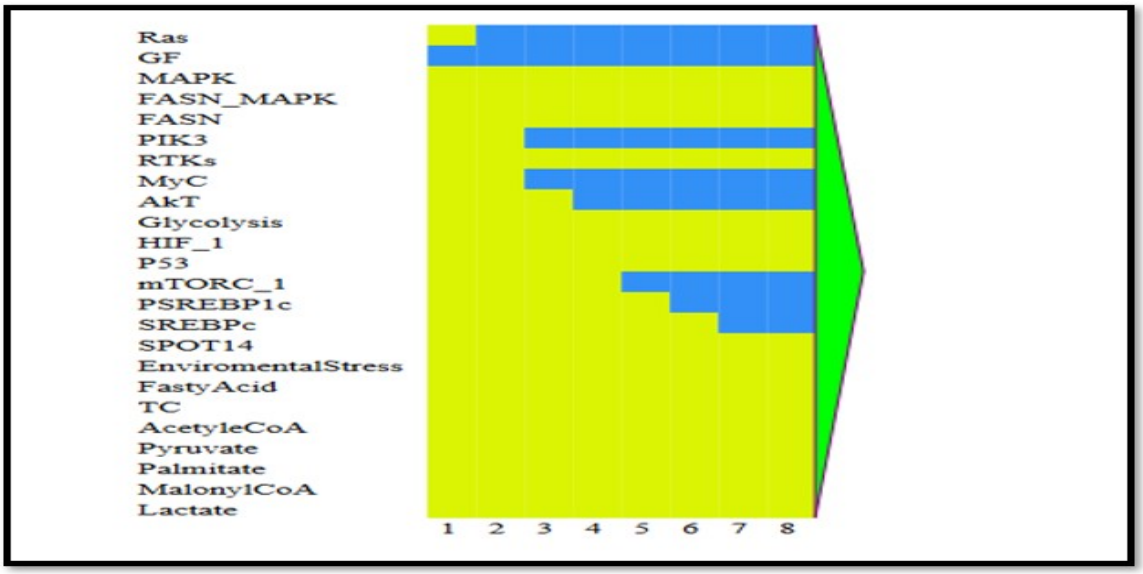

FIGURE 4.2: Protein expression pattern in glycolysis interacting FASN pathway when GF is OFF.

Similarly in Figure 4.2 the condition that are SPOT 14=ON, GF=OFF, Environmental stress= ON, MAPK=ON results in RAS, GF, PIK3, MYC, AKT, mTORC-1-1, PSREBP1C ,SREBPC, SPOT14 was OFF and, MAPK, FASN-MAPK, FASN, RIKs, Glycolysis, HIF-1, P53, ,Environmental stress, Fatty acid,Tc, AcetyleCoA, Pyruvate, Palmitate, MalonylCoA, Lactate was ON.

In the following cases we got the same result in our analysis which can be considered for not involved in malignancy of breast cancer as in their condition after

achieving few cycles the FASN pathway is recovered to its normal state. The conditions are as follows for the Figure 4.3 as SPOT 14=OFF, GF=ON, Environmental stress= OFF, MAPK=ON; for Figure 4.4 as SPOT 14=ON, GF=ON, Environmental stress=OFF, MAPK=ON; for Figure 4.5 as SPOT 14=ON, GF=OFF, Environmental stress=OFF, MAPK=ON; for Figure 4.7 as GF=OFF, SPOT14=OFF, Environmental Stress=ON, MAPK=ON.

As far as Figure 4.3 is concerned the condition mentioned in table1 results MAPK, FASN-MAPK, SPOT14, Environmental Stress was OFF and RAS, GF,PIK3, MYC, AKT, mTORC-1, PSREBP1C, SREBPC, FASN, RIKs, Glycolysis, HIF-1, P53, Fatty acid, Tc, AcetyleCoA, Pyruvate, Palmitate, MalonylCoA, Lactate was ON.

For the analysis of Figure 4.4 following nodes are GF, FASN-MAPK, PIK3, MYC, mTORC-1, SREBPC was OFF and RAS, MAPK, FASN, RIKs, AKT, Glycolysis, HIF-1, P53, PSREBP1C, Environmental Stress, Fatty acid, Tc, AcetyleCoA, Pyruvate, Palmitate, MalonylCoA, Lactate was ON. For Figure 4.5 the condition mention in Table 1result in RAS, PIK3, MYC, AKT, Mtorc-1, PSREBP1C, SREBPC, Environmental Stress OFF and GF, MAPK, FASN-MAPK, FASN, RIKs, Glycolysis, HIF-1, P53, SPOT14, Fatty acid, Tc, AcetyleCoA, Pyruvate, Palmitate, MalonylCoA, Lactate were ON.

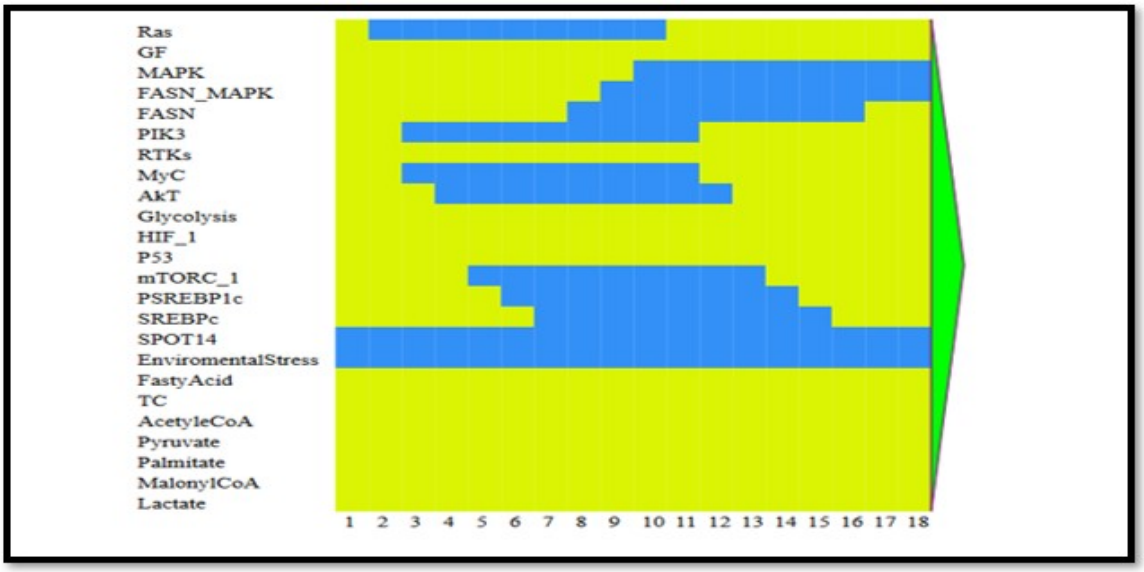

FIGURE 4.3: Protein expression pattern in glycolysis interacting FASN pathway when GF is ON and Environmental Stress is OFF.

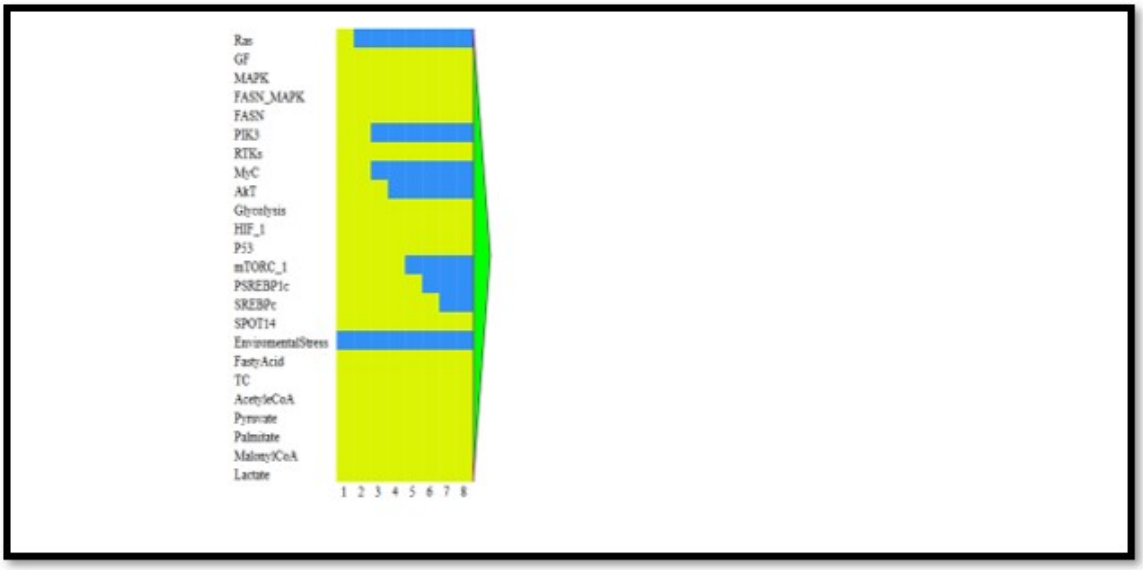

FIGURE 4.4: Protein expression pattern in glycolysis interacting FASN pathway when GF is ON and Environmental Stress is OFF.

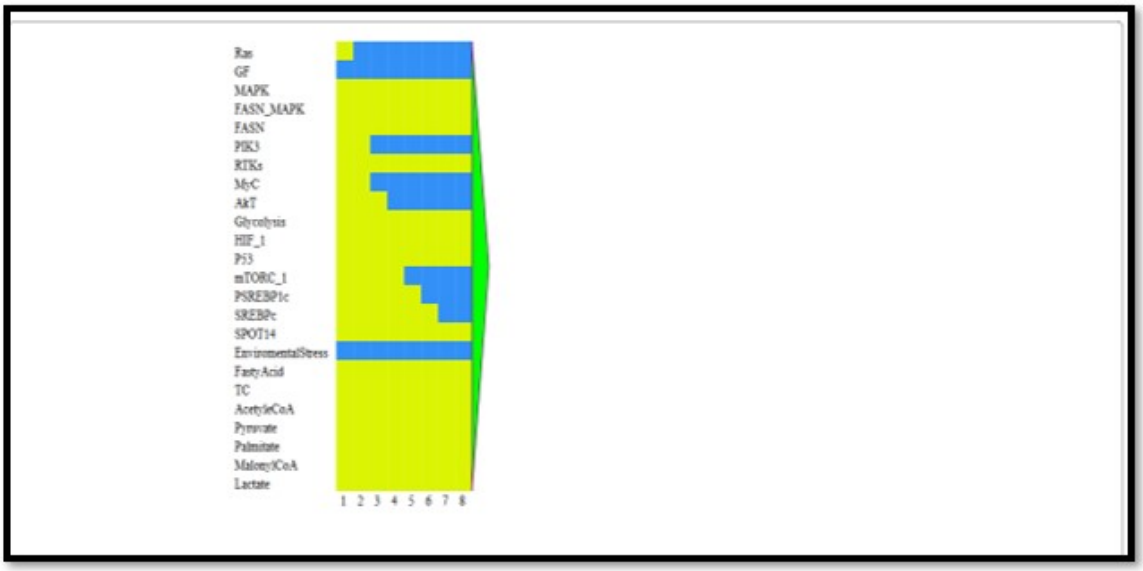

FIGURE 4.5: Protein expression pattern in glycolysis interacting FASN pathway when GF is ON and Environmental Stress is OFF, MAPK=ON, SPOT14= ON.

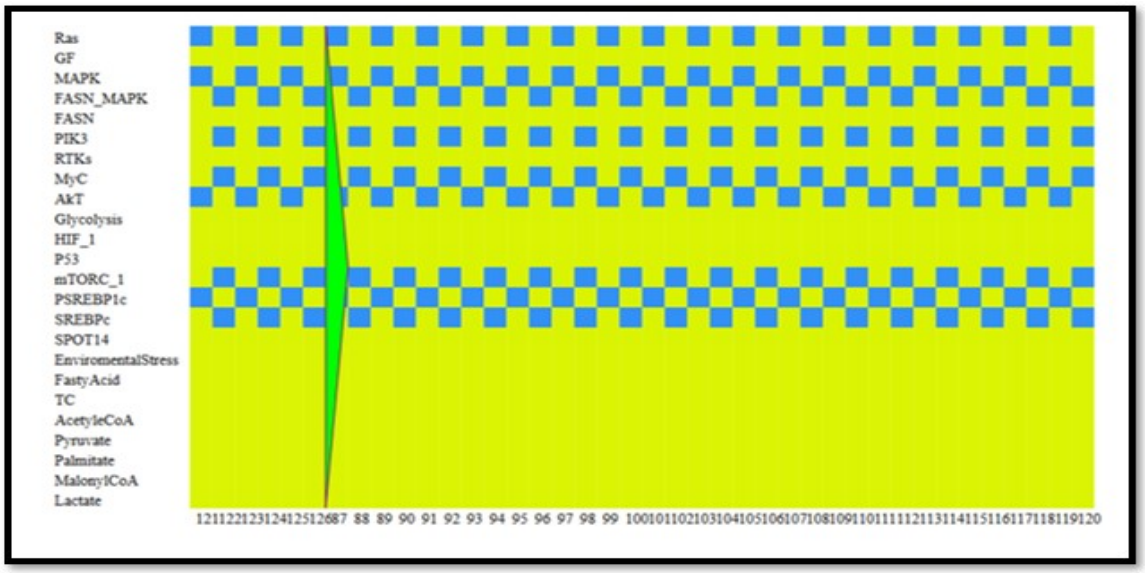

FIGURE 4.6: Protein expression pattern in glycolysis interacting FASN pathway when GF is OFF and Environmental Stress is OFF, MAPK=ON, SPOT14= ON.

There is an uncontrolled condition where we got some oscillation of nodes that could lead to the malignancies in breast cancer. GF, SPOT 14 and environmental stress are somehow affecting the cells that could be treated by cells itself. MAPK is a node that is identified in the FASN signaling pathway and is integrated with glycolysis may play important role in the breast cancer. The oscillation can be seen clearly figure 4.4 where the conditions are as; SPOT 14=ON GF=ON Environmental stress= ON MAPK=OFF. In a condition where MAPK is said of than GF, FASN-MAPK, PIK3, MYC, mTORC-1, SREBPC was found OFF and RAS, MAPK, FASN, RIKs, AKT, Glycolysis, HIF-1, P53, PSREBP1C, SPOT14, Environmental Stress, Fatty acid, Tc, AcetyleCoA, Pyruvate, Palmitate, MalonylCoA, Lactate was ON.

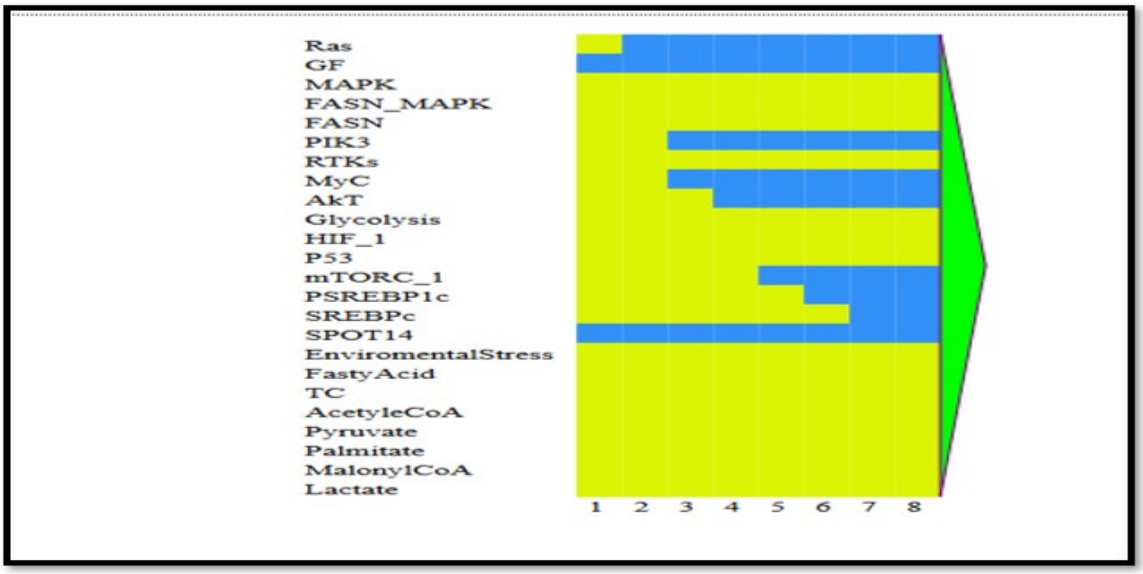

FIGURE 4.7: Protein expression pattern in glycolysis interacting FASN pathway when GF is OFF Environmental Stress is ON, MAPK=ON, SPOT14=OFF.

Females have particular vitality metabolic patterns contrasted with males [86]. Estrogens, progesterone-to-estrogen proportion and androgen levels influence the vitality material transporter and metabolic chemical articulations in cells [87]. Estrogens may expand the statement of peroxisome proliferator actuated receptor, Akt and enact AMP-initiated protein kinase (AMPK), which thusly impact the metabolic procedure, including glucose utilization, lipid up-take, capacity, lipogenesis and lipid oxidation [63]. Endocrine treatment assumes a critical part in estrogen receptor (ER) positive breast cancer treatment. Rapamycin, which represses the mTOR, is a downstream focus of Akt and upgrades the defenselessness of breast malignancy cells to endocrine therapy [88]. In any case, there is a specific extent of breast tumor patients that present with essential protection from endocrine treatment and a few patients could create auxiliary obstruction which makes it substantially harder to control the malady progress [89]. A comparative condition happens in chemotherapy and MAPK focused on the treatment of breast cancer. Subsequently, analysts are searching for new methodologies or compounds to lessen resistance against drugs and upgrade the adequacy of treatment.

Metabolic reconstruction is the essential and fundamental factor amid cell transformation [90]. External stress (SPOT14, Environmental stress, Growth factor) powers tumor cells to suit in new conditions through metabolic reinventing, caused by epigenetic change and gene transformation. Adjusted vitality digestion has turned out to be one of the signs of growth. As of late, substantially more intrigue has concentrated on focusing on metabolic proteins for malignancy treatment or switching drug opposition. Tumor cells have particular metabolic properties, including improved high-impact glycolysis, unsaturated fat synthesis, and glutaminolysis, to maintain interminable proliferation [91].

In breast cancer, numerous specialists,that objective particular catalysts in the metabolic pathways, including glycolysis, glycolysis and unsaturated fat combination, have been created or proposed. Some of them have demonstrated the capacity to upgrade the viability of current treatments and resensitize safe malignancy cells and have now been advanced to clinical preliminaries. However, to date, none have been put into routine clinical practice for a few reasons [92]. The fundamental reason might be due to a great degree complex balance of digestion and their crosstalk with other signaling pathways. Subsequently, there are three key issues that should be explained: (1) vitality pathways might be utilized by malignancy cells and additionally typical cells. The impact or harmfulness of metabolic medications on typical cells ought to be assessed close to its antitumor impact. This inquiry is conspicuous when joining metabolic medications focusing on various pathways to maintain a strategic distance from inadequate impacts or medication obstruction; (2) for breast cancer, diverse types of molecules may have a particular metabolic phenotype. FASN is the key biosynthetic chemical in the unsaturated fat union pathway that incorporates long-chain unsaturated fats palmitate from malonyl-CoA. ACC, carboxylates acetyl-CoA to malonyl-CoA. Upregulation of FASN has been accounted for both in premalignant sores and for human growths. In ordinary cells, fats are consumed uninhibitedly and FASN is downregulated, with the exception of lactating breast and cycling endometrium. The novel dissemination of FASN in various tissues makes FASN an appealing focus for malignancy

treatment. The hindrance of FASN causes unsaturated fats and gathering of substrate malonyl-CoA. Proof demonstrated that restraint of ACC did not initiate tumor cell apoptosis, which implied the aggregation of malonyl-CoA might be the explanation behind the antitumor impact of FASN inhibition [93].

4.1 Conclusion

Breast malignancy is a heterogeneous gathering of neoplasms, begins from epithelial cells and can be isolated into different phenotypes. In this work we consider how reliance upon the MAPK fluctuates crosswise over breast tumor, and characterize biomarkers prescient of pathway conditions. Directed treatment, for example, glycolysis coordinated FASN signaling pathway, has made extraordinary progress in breast cancer. Understanding the molecular pathways (glycolysis coordinated FASN signaling pathway) by which oncogenes drive malignant cell development, and how reliance of such pathways differs between tumors, could be very important for the planning of disease treatment methodologies. As of late, disease look into has concentrated on dysregulated digestion in tumor cells and metabolic reconstructing is currently viewed as a sign of malignancy. More proof of dysregulated cell digestion pathway might be related with the change in cancer treatment. In breast cancer, numerous medicines that target particular proteins in the metabolic pathways, including glycolysis and unsaturated fat amalgamation, have been produced or proposed. Some of them have demonstrated the capacity to improve the adequacy of current treatments and have now been advanced to clinical preliminaries. Notwithstanding, to date, none have been put into routine clinical practice for two or three reasons. The principle reason might be the to a great degree complex adjustment of digestion and their crosstalk with other signaling pathways.

4.2 Future Description

Henceforth, there are three key issues that should be illustrated: for breast cancer disease. (1) In its treatment diverse compounds compose may have a particular metabolic phenotype. Indeed, even a "decent" molecular sort of breast disease might be in a generally brief period thus it is basic to discover which particular compounds for particular phenotypes could be promising targets. (2) The understanding regarding particular phenotype will enable us more likely to recognize the adjusted metabolic phenotypes that may have a poorer anticipation and higher intrusiveness than different kinds; (3) it has been proposed that metabolic control may have crosstalk with FASN pathways. The hereditary controllers, for example, c-myc, PI3k/Akt/mTOR and MAPK directs digestion also FASN signaling pathways. These three issues may shape an intricate system which decides the development, apoptosis and medication opposition of tumor cells. Understanding the system for breast cancer is as yet a test for building up a fruitful metabolic treatment. All things considered, much exertion and advancement have been made in this field. Sooner rather than later, focus on tumor metabolic pathways may turn into an essential part of the extensive treatment of breast cancer.

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