CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Computational Modeling of FASN Metabolic Pathway and Drug Cocktail Design

by

Sehar Aslam

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

in the

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First of all, I dedicate this research project to Allah Almighty, The most merciful and beneficent, creator and Sustainer of the earth, He is the God the One God, the Everlasting, who has not begotten, nor has been begotten, and equal to Him is not anyone.

And

Dedicated to Prophet Muhammad (peace be upon him) whom, the world where we live and breathe owes its existence to his blessings, the pinnacle of human perfection, the scorer of humanity, the gem of mankind, the ruby of the universe, the Sultan of creation, the unparalleled, the unrivaled, the infallible.

And

Dedicated to my parents and brothers, who pray for me and always pave the way to success for me.

And

Dedicated to my teachers, who are a persistent source of inspiration and encouragement for me.

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Abstract

With the advancement of comprehension of cancer genes, focused on drugs pointed particularly at genes whose secretions are engaged in cancer pathogenesis to have reformed the idea of cancer treatment. FASN, a key enzyme for De novo fatty acid synthesis, it catalysis the Acetyl-coA and Malonyl-coA to produce Palmitate [10] but in case of normal metabolism its expression level is very low beacuse only glycolysis is enough for compensating the energy demand. Alternatively, at embryonic stage and in tumor cells FASN was found to be highly overexpressed in de novo Lipogenesis pathway for energy hemostasis in cancerous cells.Its differential expression for tumor cell survival and proliferation make it a best oncology drug target.

Metabolic pathways specifically De novo lipogenesis which is key regulators in many cancers mostly in breast cancer, are targeted by retrieving and updating through literature and a comprehensive pathway was developed based on metabolic pathways and signaling pathways, verification is given in table 4.1 and 4.2. Then with the help of Protparam tool, all physiochemical and ADME properties of all metabolites were estimated. LD50 value and toxicity were calculated by using Protox server tool and parameters for all metabolites were calculted by using these properties and parameter estimation equation of half life. As in normal metabolic pathways of cells, FASN express itself rarly because glycolysis was enough for compensating their energy demand. But in case of cancer, FASN shows its overexpression for compansating the energy demand of abnormally growing cells. So model of overexpression of FASN was developed using toolbox in Simbiology MATLAB for the simulation of over expression of FASN.

For designing a best drug with minimum toxicity the drugs already available at drugbank approved by FDA are used. After calculating all the physiochemical and ADME properties of drugs their five different combinations i.e. drug cocktails are made using Chemdraw tool. The properties of all drug cocktails calculated using different tools i.e. swissadme, ACD/I-lab reports and protox server for calculating toxicity and LD50 values of cocktails. Depending upon the fitness

value i.e. toxicity of cocktail, the best cocktail 2 choose as a drug and integrated into pathway showing up regulation of FAS gene. After the integration of dose with pathway, the expression of FAS shows down regulation in controlled simulation.

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Abbreviations

ACLY	ATP Citrate Lyase
ACC	Acetyl-coA Carboxylase
ACL	Acetyl-coA lyase
FASN	Fatty Acids Synthase
SCD	Stearoyl-Coa desaturase
NADPH	Nicotinamide Adenine Dinucleotide phospahte
SDA	Stearidonic Acid
TCA	Tricarboxylic Acid
mTOR	Mammalian Target of Rapamycin
AMPK	Adenosine activated-monophosphate Protein Kinase
G6P	Glucose 6 Phosphate
6PGSL	6 Phosphoglucono-siga-lactone
6PGDH	6 Phosphogluconate Dehydrogenase
IGF	Insulin Growth Factor
HIF	Hypoxia inducible Factor
FA	Folic acid
KG	Kitoglutrate
ADME	Adsorption Distribution Metabolism Elimination
GLS	Glutaminase lyase synthase

Chapter 1

Introduction

In the modern world, science has made extraordinary developments for the welfare of human yet at the same time there are many issues which are still challenge for the researchers. The advanced approaches, for example, Stem cell and Gene treatment and many others have demonstrated tremendous advantages in enhancing our medicinal services frameworks and revolutionized the strategies for disease treatment [13]. With the appearance of Bioinformatics, human are able for depositing the exponentially developing DNA, RNA, Protein successive data from Human Genome Project and different sources in curated databases. It additionally provided us with system and tools for analysis and interpretation of enormous biological data for their functional tasks which might be later utilized as a part of biomedical and clinical research [14]. Regardless of accessibility of such high throughput innovations, analysts stayed unsuccessful in finding the changeless cure of certain deadly diseases.

The issues made by Genome Project at the time of its completion that they would change the execution of disease therapy remained subjected to specific questions. The purpose of these questions may be the development of complex disease whose cure is challenging for biomedical scientists. Complex infections are not caused because of a single gene transformation (as if there should be an occurrence of simple disease) rather than they are controlled by polygenic (Multiple qualities) factors alongside some environmental elements, the way of life are also heritable in

nature [22-23]. In such diseases, hereditary elements contribute mostly to disease hazard and they don't show heredity pattern. This environmental gene expression encourages in conferring better knowledge of infection causal and later aides being developed for targeted treatment [56]. The most critical case of complex infection is cancer which is characterized as the uncontrolled/unusual expansion of cells because of mutation in certain gene under the control of environmental or heredity factor. Cancer is intricate as in it includes a progression of connection of hereditary and environmental factors that straightforwardly deregulate different components of the human body, for example, Immune system, DNA Repair strategies and apoptosis and so on [33]. These systems comprise of different signaling pathways so they in collaboration with epigenetic forms decide the phenotype of the tumor [32].

Latest cancer genome studies have prompted the recognition of various pathways related to the tumor [169,156]. Due to extensive mutations in these particular genes incidence of cancer and development had been examined, consolidated combined for facilitating cancer phenotypes. Moreover, advances "NGS" has empowered the selection of various malignancy sorts and its further types, revealing both intra and inter tumor heterogeneousness [170]. In spite of the huge variety of abnormal cell progression, neoplastic events are combining to change cell metabolism in abnormal cells. No doubt, study of cancer cells have revealed a metabolism that is not the same in abnormal cells as compare to the metabolism of normal cells because of extra demand of energy, fatty acids, and proteins and all fundamental needed for development [72]. This crucial ability of cells of the tumor has prompted improvement of the many major treatments with methotrexate and chemotherapy, as of now in the middle of 1950 [53], trying to aim abnormal growth in cancer. These medicines which meddle with the utilization of FA (folic acid) in cells of tumor have used against metabolites for blocking DNA inhibiting the formation of DNA and development of the tumor. Late prompted the acknowledgment of modified metabolism in tumor as a cause of cancer has become confirmed factor [47]

Metabolism of the cell has excellently regulated with coordinating signs received by factors of intra and extra-cellular environment of the cell. The switch of metabolism pathway advancing uncontrolled development has regularly activated with the help of transformations in signaling pathways which stay at the essence of balancing of energy and anabolism, for example, Hypoxia-inducible factor 1a, mTOR, AMPK and PI3K [82,126,174,63]. Pathway with mutation leads to constitutively active developmental signals that cause cells to multiply wildly. Along with the intracellular hereditary modifications, the strange environmental conditions also assume an important part in changing cell metabolism, pH levels, heterogeneity in oxygenation and nutritional accessibility have combined with characteristically alter cancer cells development, improving with persistent access of growth components and ability of redox that enable tumor survival and multiply in unfavorable particular pressure [37].

Lately, research has an essentially improved concept of the hereditary and molecular events fundamental of the metabolic useful phenotype of cancer cells. The growth of gene sequences and gene methylation pattern, protein, genes and microRNA expression estimations, and also metabolites consideration, have uncovered a far-reaching and picture of abnormal cellular processes [113]. In any case, the whole metabolic system is involved, with a couple of thousands of biochemical changes. To understand broadly how the different cell segments connect with each other and to additionally figure how the metabolic system reacts to various hereditary and environmental disturbances, computational approaches are employed. Specifically, computer models empowering the examination of the condition of networks at various stages and at the genomic level have become useful for both non-cancerous and cancer cell metabolism, and also to improve the capacity of distinguishing effective medication, biomarkers and drug targets [119].

With the advancement of comprehension of cancer genes, focused on drugs pointed particularly at genes whose secretions are engaged in cancer pathogenesis to have reformed the idea of cancer treatment [117]. This technique has created amazing single-molecule therapy for single-molecule directed treatment, demonstrating either transient advantages or no advantage at all. This requires pathway directed

therapy that uses numerous molecules. Cancer proliferation is a multistage procedure including several overexpressed or dysregulated genes that underlie the cell signaling systems [113]. Mostly cell signaling systems are dynamic and nonlinear. It is not obvious how drug cocktail to be formed with a specific end goal accomplishes maximal efficacy. It is harder to acquire a low dose drug with insignificant adverse effects and medication protection, which requires the constituents of the medication are balanced in order to acquire extreme synergistic impact [97]. The solution for such issues requires complex computational demonstration and investigation. The computational model would then be able to be modulated in distinct approaches to test distinctive drug strategies. These strategies provide experiences that how a drug target ought to be wired into the control component of the system. This approach, network modeling mathematical analysis drug discovery, may turn into the treatment [88]. Molecularly focused on therapeutics give possibly more reliable expressions while significantly diminishing toxins as compared to chemotherapy. For cancer signaling networks which are typically intricate, different molecules should be focused so as to remain tuned in to the control components of the network and to accomplish the greatest synergistic impacts. Mathematical modeling and computer based simulations are essential in imitating the progression of the network, some of which may respond to normal or cancer phenotypes [121]. Most critically, the impacts of numerous molecules can be signaled by perturbing numerous parameters in the model. As compare to the modeling of microorganisms two critical focuses are considered while using these human reconstructions (i) Models are not specific for a type of cell and tissue. They include all potentially happening reactions in metabolic pathway of human, their solution consists of various possible practices which have additionally controlled to accomplish stage of tissues and sensitivity of cells with respect to metabolism (ii) the role of various cells of human and tissues has harder to decide or maybe not possible, particularly in non-proliferating part of tumor (and henceforth maximal biomass yield can't be accepted) [122].

1.1 Problem Statement

Computational Modeling and Analysis of FASN in lipogenesis and to design a pathway directed therapy with maximal efficacy and minimal toxicity .

1.2 Proposed Solution

Elucidation of quantitative insight of the pathway most critically involved in tumor formation and progression and consensus of the altered cell behavior for determining the site at which oncologist should intervene is of prime importance from therapeutic point of view and analyzing the role of FASN and other important protein of lipogenesis pathway in Breast Cancer through Mathematical Modeling and simulation in MATLAB.

1.3 Objectives

The objective of this research is:

1. Computational modeling and analysis of FASN in the lipogenesis pathway.
2. To Model a pathway directed therapy with maximal efficacy, minimal resistance and reduced toxicity.

1.4 Scope

A perfect molecular target should especially show its expression or activation in cancerous malignant cells. There are two distinctive qualities of FASN that has made it appropriate for being an antitumor target on its tissue circulation and its enzymatic functioning. FASN exhibit high expression in breast tumor however not in non-lactating typical breast tissue utilizing FASN as an objective will impact

the multiplying part of the breast while the non-proliferating compartment will stay unaffected. FASN particular restraint, C75, and EGCG are notable cases of inhibitors. Though, their remedial efficacies are restricted due to either their high harmful level or temperamental nature. Thus, looking for a more steady and intense therapy that is an objective inhibitor will be an imperative future pattern for innovative work for oncologists.

Chapter 2

Literature Review

2.1 Metabolic Shifts Linked to Cancer

Yizhak [32] initially give a concise diagram of the metabolic adjustments to happen in malignancy (cancer) [37,28,73]. Prominent climaxes of tumor breakdown were found by Otto Warburg, demonstrating that growth cells use glucose amount and discharge it as lactate with the availability of oxygen, a mechanism introduced at high-impact known as "glycolysis" or the "Warburg effect" [12]. In comparison to the ordinary cells utilizing glucose of mitochondria by means of the tricarboxylic acid in TCA cycle, this sensational increment in glucose utilize by tumor (cancerous cells) has manipulated at clinics stage to imagine disease by (18F)- 2-deoxy-D glucose positron outflow tomography (FDG-PET) [172]. From late revelations, glycolysis process in diseased cells has been contemplated broadly and a few glycolytic responses were observed to be key controllers of tumor breakdown (as shown in fig.1). Past the Warburg impact, real changes in disease have been recognized pathways engaged with the generation of key biomass fragments. The uncontrolled multiplication in the cell of cancer and proved by anti-metabolite based chemotherapy, synthesis of undeveloped delay in the amalgamation of nucleotides, and NADPH by the oxidative "pentose phosphate pathway" (PPP), expanding with glucose break down), have basis for efficiently increasing cells. The step of

glycolysis, another pathway is the synthesis of serine that has become vital for amino acids, lipids, and blends of nucleotides. The high regulation of pathway is interconnected to the capacity of best growth tumor to metastasize [65]. Moreover, genome studies have revealed that serine production is the substantial cause for the propagation of many cancer cells. The genes for the phosphoglycerate dehydrogenase (PHGDH) which is the enzyme that catalyzes the production of serine, exceptionally communicated in a few tumors, and melanoma and breast disease cells with PHGDH enhancement occupies huge glucose related carbons into glycine and biosynthesis of serine [161,167]. Numerous tumor cells experiencing oxygen-consuming glycolysis require carbons of glutamine to recharge the TCA cycle and support speed up anabolism. For cells, Glutamine is also a critical nitrogen hotspot (80). Two glutaminases GLS1 or GLS2 can deaminate the glutamine by one of the creating glutamate and alkali. In a few situations such as deficiency of oxygen (hypoxia), glutamate produced from a-KG can experience reduced carboxylation to produce oxaloacetate, acetyl-Co, citrate, and to help anabolism without oxygen [164]. Glutaminase had shown overexpression in various cancers, and its hindrance defers cancer development [159,174]. The metabolism of cancer has non-restricted to the adjustment of metabolic to ecological changes or increase expansion levels. Transformations influencing important pathways of metabolism have been revealed in genetic types of disease or appeared to expand tumor inclination, uncovering that different digestion could likewise be, now and again, the reason for growth. Not long after this original interpretation, fumarate hydratase (FH), the catalyzes the proselyte's fumarate to cancer, have discovered transformed in inherited leiomyomatosis and growth of Kidney cells "HLRCC" [160,79]. Transformations in the Tricarboxylic Acid cycle, its compounds force cells to depend on mutated products of TCA cycle and to amass the high amount of fumarate and succinate. This has believed that subsequent adjustment of subordinates of oxygen easily altered type of the HIF, with the availability of Oxygen, offers a vigorous expression of glycolysis and increase to pseudo hypoxic. Another important compound of TCA cycle is isocitrate dehydrogenase (IDH) that observed to be altered in disease cells.

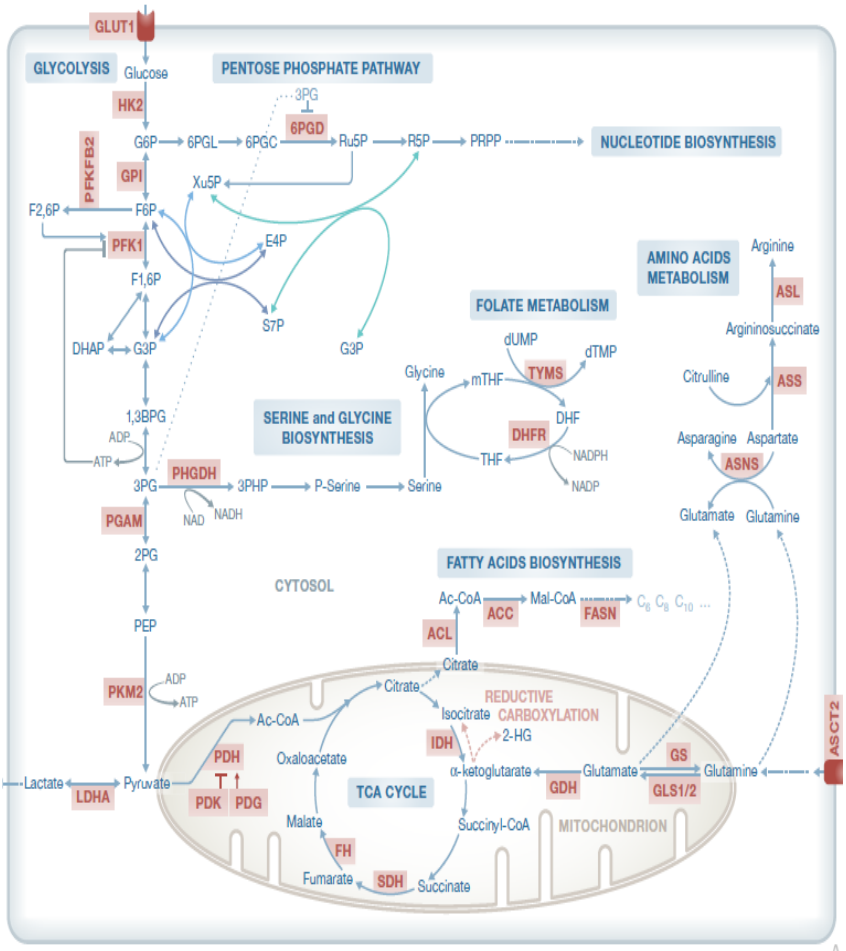


FIGURE 2.1: Central Metabolic Pathways and their association with key metabolic enzymes.

Heterozygous transformations in the dynamic site of IDH1 and IDH2 isoforms were examined in the high degree of low-quality glioma and intense myeloid leukemia (AML) patients [194,61,168]. Mutation in IDH has not only reduced the capacity to change isocitrate to a-ketoglutarate but it also reduces the production of 2 hydroxyglutarates (2HG) by utilizing a-ketoglutarate (143) and that is the numeric condition in AML and glioma. Particularly artificially synthesized inhibitors for the mutation in IDH1 and IDH2 have at trials in clinics [152]. By bringing together all facts, 2HG, fumarate, and succinate have been named as 'onco-metabolites' offering that there exist some distinctive oncometabolites and suspect disclosure the probability that diverse oncometabolites exist and foresee divulgence.

2.2 Tumor Metabolism as a Therapeutic Target

As the discovery of new targets is one of the fundamental objectives of metabolic displaying in disease, the considerable number of deregulated metabolic pathways gives the chance to focus therapeutically on these pathways. A noteworthy test has that most by far of pathways of metabolism utilized by disease cells are likewise basic for the persistence of ordinary cells, indicated by the unfortunate reactions of a few chemical therapy specialists. In any case, the nearness of tumor-particular chemical subtypes or alternation in the action of a pathway may permit special focusing of growth cells. The helpful impacts of focusing on a few metabolic proteins have been explored. For example, glycolytic inhibitors, for example, GLUT1inhibitor and 2-deoxyglucose experienced trials in clinics [146,10,45]. And the impact of them, however, have observed to be constrained, possibly because of the solid increment in glutaminolysis showed by a few cancers, and the capacity of cancer cells to deliver ATP by oxidative phosphorylation with useful mitochondria. A few inhibitors of amino corrosive digestion have likewise been examined. Glutamine, the primary focused amino acids that can be extracted from the tumor patient's blood. Phenylacetate lessens glutamine accessibility in this way hindering disease cell multiplication and advancing separation [166,174]. Glutamine's exclusion straightforwardly from blood may likewise expand the level of the body drains its specific particular storage of muscle (cachexia), other technique to target GLS specifically [191].

This has prompted the utilization of asparaginase and for the treatment of infancy acute lymphoblastic leukemia, the enzyme that changes asparagine to aspartate and ammonia [157,183].Going besides amino acids metabolism, a few blocking agents of fatty acid formation have examined and produced. TCA cycle derived citrate and NADPH synthesized endogenous fatty acid, which can be delivered by different catalysts and PPP. In the cytosol, ACL converted the citrate into oxaloacetate and acetyl-CoA [62]. Production of fatty acid initiate with enzymes ACC produced the malonyl-CoA by changing over acetyl-CoA and has been trailed by the progression of steps by which malonyl-CoA and acetyl-CoA are changed

into palmitate by FASN. Numerous tumors in this manner express the excess of fatty acid synthase, for example, colorectal, breast and endometrial cancers (34), and inhibiting factors of FASN may destroyed cancer cells specifically and make them sensitive for different treatments, for example, Herceptin [201,86,73].

Going other than amino acids metabolism, a couple of fatty acids inhibitors arrangement has been created and contemplated. NADPH and TCA cycle derived citrate generate Endogenous fatty acids, which can then be conveyed by the diverse catalysts and PPP. Citrate is changed to acetyl-CoA and oxaloacetate by ACL when it is present in the cytosol. The formation of Fatty acids starts when ACC is changing acetyl-CoA to malonyl-CoA and this was followed by the movement of ventures where acetyl-CoA and malonyl-CoA are changed by fatty acid synthase (FASN) to make palmitate.FASN is overexpressed in various cancers by this way i.e colorectal, endometrial and breast cancer [34], and FASN inhibitors are either destroyed cancer cells particularly to various medications, for instance, trastuzumab and 5-fluorouracil (Herceptin) [83,196,201].

Different inhibiting enzymes of de-novo lipogenesis, for example, monoglyceride lipase (MGLL), ACL, ACC, choline kinase and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), have demonstrated successful for therapy of cancer at preclinical practices and the enzymes have the concentration of improvement in drug, in fact few of them, such as statins, are undergoing subordinate in clinical trials recently [83-84,88,62,90].

2.3 Human Metabolism Modeling

At human metabolism, Genome-scale metabolic modeling of (GSMM) has been remade to speak to gathering for metabolic responses known to happen in cells of human [163,187,40,57-58].

The given models are used to display both healthy and unhealthy metabolism of human, which is extensively surveyed by Mardinoglu and Nielsen 2015, Bordbar and Palsson 2013 [35,59]. Considering these difficulties question is then how Yizhak [32] might use these recreations to study about normal and infected human metabolism.

TABLE 2.1: Rearrangement of Human models and their utilization in metabolism of cancer (Keren et al., 2015)

Cancer Type	Application	References
Genetic	Studying the association between cell proliferation and the Warburg effect	[178]
Generic	Pathway contribution to NADPH production in cancer	[177]
Generic	Identification of cancer selective targets	[178]
Generic	Predicting combinations of anti-cancer drugs with minimal side effects	[42]
26 tumor tissues	Identifying cancer-specific metabolic Pathways	[7][10]
Liver Cancer Cell Line	Identifying P53-associated metabolic Changes	[47]
The NCI-60 cell line collection	Studying the association between cell proliferation and nutrients uptake rates	[40]
Breast Cancer	Studying the metabolic differences associated with tumor stage and type	[49][50]
Clear Cell renal cell carcinoma	Identifying synthetic lethal interaction in FH-deficient cells	[44]
The NCI-60 cell line collection	Predicting drug-reaction interactions	[54]
The NCI-60 cell line collection and breast-/lungs cancer clinical samples	Personalized prediction of metabolic phenotypes and identification of selective drug targets	[77]

The NCI-60 cell line collection	Association of the Warburg effect with cell migration and identification of anti-migratory drug targets	[78]
Hepatocellular carcinoma	miRNA was simulated to predict their ability to reduce cancer cell growth	[185]
Colon and breast cancer cell lines	Metabolomic network correlations	[53]
Nine cancer types (TCGA/CCLE)	Identification of oncometabolites	[55]
16 cancer tissues	Identifying cancer-specific metabolic features	[58]
Breast, bladder, liver, lung and renal cancer	Topological analysis of ccRCC-specific metabolic processes	[45]
Hepatocellular carcinoma	Personalized model reconstruction and selective drug target identification	[34]
15 cancer cell types	Studying the topological features of anti-cancer metabolic drugs	[186]

2.4 Modeling of Environmental and Genetic Stresses

At the intracellular metabolite level, there is another kind of perturbations, in which a deficiency in metabolite is stimulated by the reduction of the system [112]. Stage of metabolism of cells can be reassessed by distinctive mixes.

Notwithstanding, alternative target functionality have been connected in a way that cell tends to deviate from their past abnormal kind condition [165,177]. Inquisitively, it has been exhibited that during the guideline approach shows the after effect of excess genetic stress, the other one is more susceptible for those

cases that don't have any framework for alternative regulation for the optimal development arrangement [164-165].

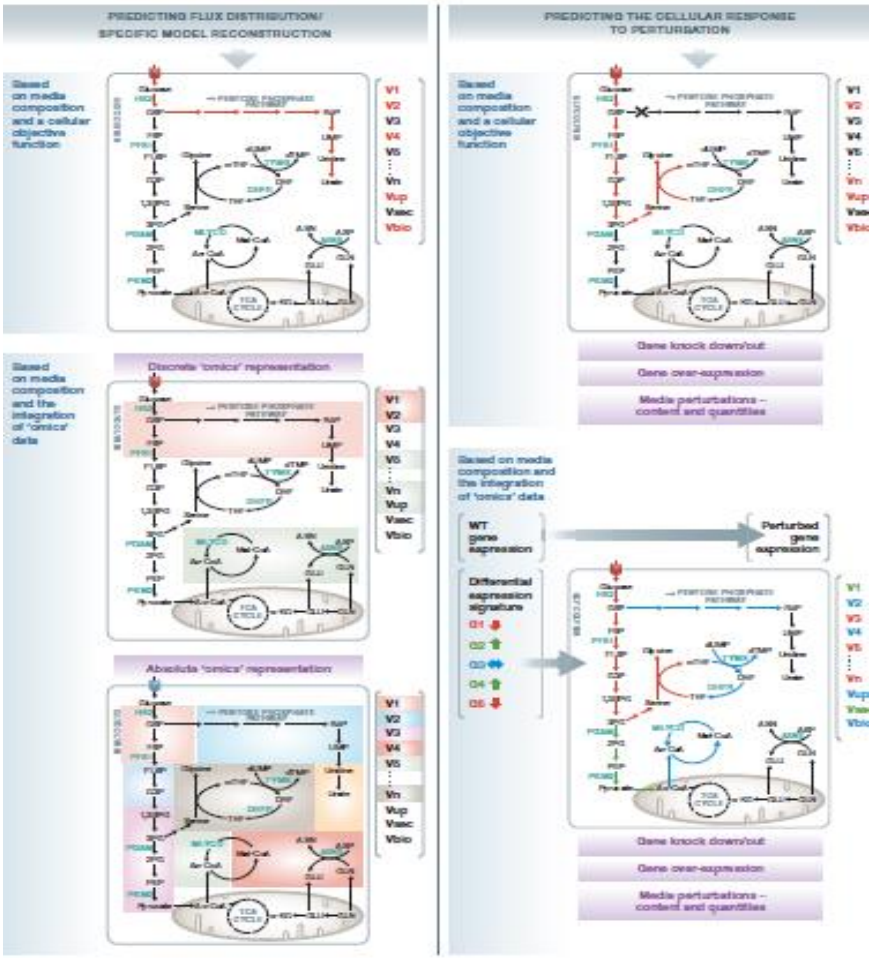


FIGURE 2.2: Genome-scale metabolic modeling as a platform for predicting flux distribution and simulating cellular perturbation (Keren et al., 2015).

Regardless of any condition-specific high throughput data, they have been continuously ustilized for many drug disclosure applications [167,184,178], and furthermore building of products of metabolism [136-137], simulation of reductive development [76,192,193] essential expectations of genes [41,190,187]and some more [193].

Regardless, the period of vast scale omics information gives a chance to decide the disturbed stage with no need to accept an early characterized target purpose. Scientists have designed another algorithm that uses source and gene expression of target provide information to predict perturbations that are well on the way to change the metabolic state from one to another. This solution has become useful

for examining living things maturing and prompted the distinguishing proof of vital life expectancy expanding part of DNA.

Latter, [33] have explored antimetabolites expecting to on numerous enzymes at the same time. Implement that technique for customized 6 models of patients suffering from hepatocellular carcinoma has anticipated it active opposite to metabolites. Other than these l-carnitine analogs had examined experimentally by checking the effect of its inhibiting agent carnitine palmitoyltransferase as the multiplication of a cell line "HepG2", demonstrating the diminished ability of tumor.

Models that are based on the cell of a couple of many usually multiplying and malignant division of cells have been worked by the quantitative incorporation of their gene activity range. These specific models of the cell were then appeared to effectively predict metabolic expression at a personal level, including the level of cell development, reactions of drug and its biomarkers. These models have been additionally utilized for distinguishing specific targets of the drug, in which it has prompted the laboratory approval of a best expected particular target, in cancers of white blood cells and cancerous kidney cell division versus their ordinary amount.

These models of cancer have been used to anticipate the proportion amongst glycolytic and oxidative ATP generation rate, demonstrating its positive relationship with the migration of cell. Following, twelve of novel genes issues that were explored to diminish the percentage were discovered practically to the importantly decreasing migration of cell, while having no impact on the development of the cell, by discovery. Imperatively, these issues may decrease cell toxin concerning clonal choice of cancer cells and the probability of development protection.

The different medication targets already exposed by genome-scale modeling based investigations and more approved experimentally affirm of capacity to catch system scale range impacts of these couldn't have been distinguished by data examination alone.

As numerous studies have focused on forming cancerous cells in vitro apart of their tumorigenic environment, presently it is being accepted largely that the tumor microenvironment plays an important role in reconstructing and characterizing metabolism of cancer cell [60]. The in-silico study between the relationship of a cell and tissue by the help of GSMMs have shown both human tissue and microorganisms, but it has not yet been studied in context with the cancer cell and supporting cells in their surrounding system [54,76]. Showing a versatile transfer of materials in-between different cells can take us to a more closure and exact demonstration of tumors in-vivo and exposure of more closely related phenotype metabolically couldn't have been found without the demonstration of every single cancer cell alone. Tumor cell is also exposed to changing the pH and oxygen levels while connecting with another cell in their microenvironment [7].

Previous studies have focused on forming in vitro cancerous cells apart of their cancerous environment, presently it is being accepted largely that the tumor microenvironment plays an important role in reconstructing and characterizing metabolism of the cancerous cell [154,179,175]. The in-silico study between the relationship of a cell and tissue by the help of GSMMs have shown both human tissue and microorganisms, but it has not yet been studied in context with the cancer cell and supporting cells in their surrounding system. Showing a versatile transfer of materials in-between different cells can take us to a more closure and exact demonstration of tumors in-vivo and exposure of more closely related phenotype metabolically couldn't have been found without the demonstration of every single cancer cell alone. Tumor cell is also exposed to changing pH and oxygen levels while connecting with another cell in their microenvironment.

All these elements play an important role in the proliferation of tumor and are also known to influence metabolism in tumor cells [158]. By means of GSMMs, the oxygen and nutritional accessibility can directly be stimulated. Environmental factors like pH for modeling are less straightforward. One methodology that is possible for solving these issues is by applying investigation methods on the structural basis to forecast the influence that has been started by the level of pH over the functioning of metabolic enzymes. Interestingly, a concept that resembles the

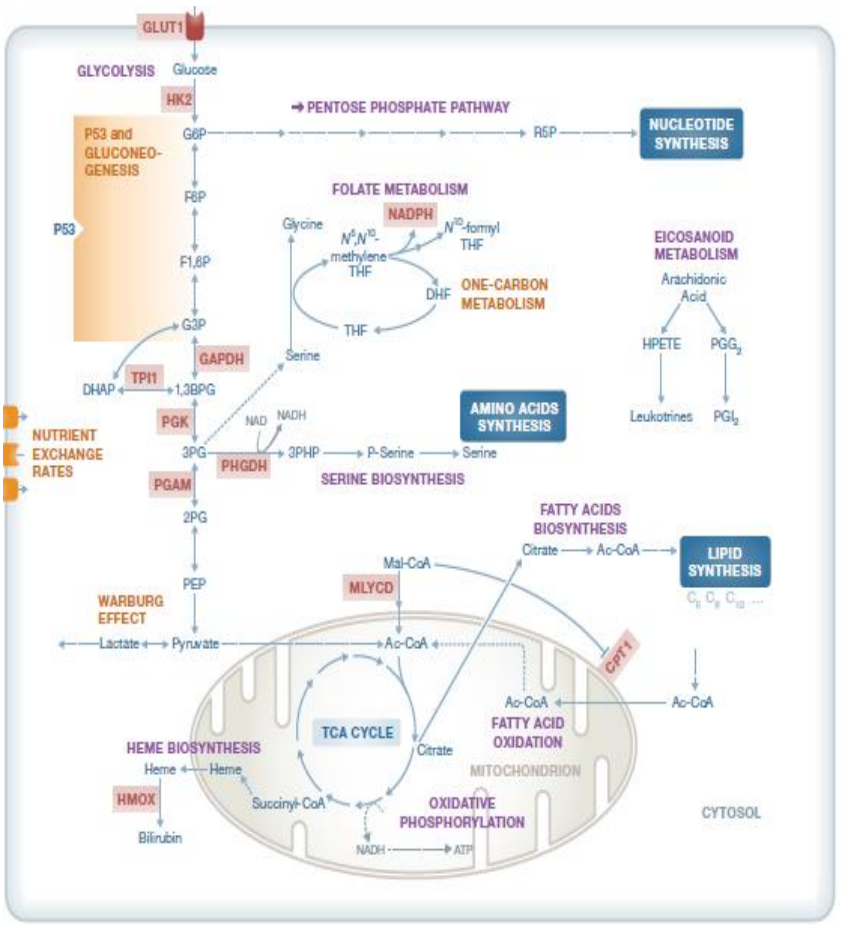


FIGURE 2.3: Metabolic processes, enzymes, and metabolites studied through genome-scale metabolic modeling (Keren et al., 2015).

examination has been used to study the reaction of Escherichia coli to different temperatures that expose the protein functioning which controls the network work at higher temperatures and hence gives a mechanistic analysis of mutations that have been found in strains which are adapted to the hotter environment [10].

Resistance to chemotherapy treatments is a noteworthy issue faced by the recent cancer biologists, and systems for its achievement have different ways [26]. Genome-scale modeling is used in this context to distinguish the unrestrained function of existing digestion protein, thus exposing elective pathways enable for bypassing reactions of oncogenes. To distinguish gain from work in the mutation of enzymes and improve our concept of enzymes' catalytic side functioning this approach can be used [27-28]. The unrestrained function of metabolic enzymes has already been considered by genome-scale modeling of Escherichia coli, both

exploring the basic characteristic of these enzymes and recognizing vital metabolic networks that develop precursors for cell development under different environmental conditions. The GSMM framework also facilitates the simulation of various perturbations at the same time and encourage by examination of combinations of the drug for treatment and SL based therapies. Their results give a chance to accomplishing more noteworthy efficacy offering huge potential for enhanced prognosis [29]. Shortly, one can take note of that besides to GSMMs, other earlier methodologies used in the modeling and simulation of biological procedures, which include expensive binary networks, the greater and small-scale examinations through standard ordinary differential conditions (ODE) [30,71].

Yizhak concentrates how genes guide their function in different biological conditions, implemented to distinguish targets of drugs at various levels, including brain, breast and cervical carcinoma [32]. In other way, Boolean network investigations include the demonstration transcriptional regulatory and signaling pathway and networks and were utilized for distinguishing genes driving the mutation b/w various tumor development events [61,97], and deciding controlling mutations that enhance cancer phenotypic changes as an expression of the surrounding of cell. Differential networks were basically utilized as a part of the list of concentrate changing aspects of cancer disease development and understanding disease reaction to treatment[173,188].

2.5 Background of Disease

Breast Cancer is the most predominant type of cancer and the second driving reason for mortality in women around the world [1]. As per the World Cancer Report [2], breast cancer includes 22.9% of all growths in women with an expected 1.4 million new cases every year, bringing about more than 458,000 passings in 2008 [2,3]. It was assessed that more than 1.6 million new instances of breast cancer malignancies developed worldwide in 2010. In other Asian countries and Malaysia, the prevalence of breast cancer is on the expansion, indicates changes that are known

hazard for the breast growth. This term alludes to numerous natural impacts, that are familial risk factors for breast cancer malignancy to incorporate; weight and intake of dairy products as a food in significant amount, no and less breastfeeding [11]. The Warburg impact portrays the marvel by which diseased cells depend on vigorous glycolysis for vitality as opposed to oxidative phosphorylation [12]. The Reverse Warburg impact depends on the perception that the non-malignant tissue, to incorporate fibroblasts stromal, encompassing malignancy cells additionally utilizes oxygen-consuming for energy through glycolysis.

Already proposed that fibroblasts have attempt vigorous glycolysis "invigorate" by epithelial tumor cells the and along these lines discharge the items pyruvate and lactase. And these metabolic products would effectively "sustain" the malignancy bringing about expanded expansion. One examination revealed that stromal tissue in breast cancer had the Reverse Warburg impact, for example, irritation and markers of oxygen-consuming glycolysis [14].

Despite the fact that Breast cancer is viewed as a hereditary ailment in which a few transformations and genome dynamic changes are available [15] late research are adapted to attempt and comprehend different systems adding to the (arrangement) advancement and movement of the disease [18].

Various features are related to breast cancer are age, hereditary qualities, and distinctive ecological elements. Most breast cancers basically influence ladies matured 50 and more seasoned, there is a reasonable connection amongst menopause and breast cancer rate [3-5]. Other metabolic procedures add to the arrangement of an ideal microenvironment for the treatment of breast cancer, In the most recent century, to be specific, mastectomy, chemotherapy, and radiotherapy, or a combined therapy [3]. With the quick advancement of sub-atomic meds, novel remedial methodologies, for example, hormonal treatment and sub-atomic focused on treatment, have been proposed to enhance clinical result; be that as it may, the result of such methodologies is as yet not perfect [3,4].

The diverse atomic different types of cancer in breast emulate the statement of particular gene expression In breast cancer its luminal subtypes have tried to

show hormonal receptors and abilities of luminal epithelial cells, for example, articulation with less atomic weight cytokeratins; the overexpress HER2 shown by ERBB2+ breast cancer and the 'typical breast disease' sort have revealed the expression of genes related to non epithelial cells and fat cells, for example, integrin α and lipoprotein lipase [9].

2.6 Metabolic Pathways Involved in Breast Cancer

Metabolism is a procedure whereby biochemical, oxygen, and supplements are employed to create energy as ATP expected to perform cell works or used for macromolecular synthesis. As of late, metabolic exercises have reemerged as a procedure ready to produce other various cell reactions [5].

In breast tumors metabolism, similar to most cancer, intensely depends on the utilization of oxygen-consuming glycolysis and glutamine catabolism to help cancer development. Both pathways are imminent and focuses on breast tumor treatment [15]. Vigorous glycolysis sidesteps mitochondrial oxidative phosphorylation to keep away from a lopsided and negative overproduction of ATP and NADH. Like glucose, glutamine is taken up by tumor cells and has an essential part in the recharging of the mitochondrial citrus extract carbon pool. To expand the proliferative movement tumor cell ordinarily needs to adjust its metabolic pathways offering to ascend to a metabolic reinventing which is by and large clarified by the metabolic move from mitochondrial oxidative phosphorylation (OXPHOS) to oxygen-consuming glycolysis (Warburg effect) [17][18][19].

Postmenopausal women with a weight record (BMI) of more than 30 have a 31% expanded suspectability breast cancer contrasted with postmenopausal women with a BMI underneath 25; obese ladies who create [21]. One investigation checked on the confirmation of the relationship amongst overweight and breast cancer and

proposed the contribution of a few pathways. Adiposity can increment coursing insulin levels and insulin-like growth factor-I (IGF-I), tumor necrosis factor-α (TNF-α) and adipokines, for example, leptin [9].

2.6.1 Glucose Metabolism in Breast Cancer

Cells are profoundly composed and a consistent supply of energy is required to make and keep up the organic requests that keep them alive. This energy is obtained from the cleavage of put away in sustenance particles, which fill in as fuel for cells [54]. In recent research have depicted basic pathways of metabolomics in breast cancer and portrayed on metabolites that cause tumor development and movement. Late advances propose that metabolic profiling gives new chances to enhance results in breast cancer. Rather, oncogenic MYC and the TP53 tumor silencer gene appear to affect metabolism in breast cancer. In breast cancer, lactate (as a finished result) and glutamine (as a substrate) upgrade disease aggressiveness and constitute targets focus on breast cancer treatment [30].

Energy homeostasis of an ordinary cell is adjusted by no less than three metabolic pathways i.e. lipogenesis, glycolysis and tricarboxylic (TCA) cycle, and these pathways are firmly connected to amino acid and additionally nucleotide biosynthesis [24]. Ordinary cells use an assortment of energy, for example, glycogen, unsaturated fats and amino acids, glucose is considered as important energy hotspot for the development of cells. Glucose has transported by the glucose transporter framework and through the glycolysis pathway has changed over to pyruvate [44]. After that Pyruvate changed over to acetyl-CoA and in mitochondria used as a substrate for the TCA cycle. It has for some time been perceived that the tumor cells need increase level of energy metabolism due to their dynamic expansion and proliferation [64]. Due to similar issues, tumors turn out to be extra hypoxic and subsequently they have to depend on non-oxidative energy resource, for example, glycolysis as initially announced by an effect of Warburg. Then again, in cancer cells, more lipogenesis is by all accounts causative both to producing energy (beta-oxidation) and building mass (cell film and so on) [70]. The rate of lipogenesis is

additionally essentially quickened in tumor cells with a specific end goal to make up for the higher rate of expansion. It is progressively obvious that numerous genes engaged in metabolic pathways assume coordinate parts in tumorigenesis and tumor development [29].

Glycolysis is a process of catabolism that produces two pyruvates by changing molecule of glucose with the production of ATPs and two reduced NADH particles [65]. In the oxidative phosphorylation pathway in the presence of oxygen pyruvate experiences oxidation to produce CO2 and H2O, bringing the generation of around 36 molecules of ATP. Formation of Lactate from glucose in the presence of oxygen is Warburg effect [12]. Increased glycolysis is taken as to the seventh sign of cancer [55]. Multiplication of Ordinary cell in tissues is managed by the accessibility of development regulating factors and its interaction with cells from outside. In initial cancer stage, an uncontrolled division of cells from blood transfer cancerous cells far from veins thusly, by nutritional and supply of oxygen [58]. Cancer cells have a significantly utilization of glucose by the pathway of glycolysis in which pyruvate does not transfer to the Krebs cycle i.e. the oxidative phosphorylation pathway that suitably produced lactate by changes over pyruvate: the purported Warburg effect. The process of glycolysis happening in cells of growth not only linked with a decrease in Krebs cycle [13]. The Reverse Warburg impact depends on the perception that the non-transferable cancer cells i.e. benign tissue, to incorporate into fibroblasts stromal, encompassing tumor likewise utilizes oxygen for energy through glycolysis [89]. It is hypothesized that tumor cells of epithelial "fortify" the fibroblasts to embrace glycolysis with oxygen and consequently secretes the pyruvate and lactase. These products would as metabolites successfully "nourish" tumor cells bringing about expanded development. One investigation revealed cancer cells of breast tissue of stroma have highlights of opposite impact of Warburg, for example, irritation and glycolysis markers [9].

Rapidly developing cancer cells experience the lack effects of an absence of oxygen and nutrients because of the dispersion furthest reaches of supply in blood, and hence, persistent metabolism of glucose and lactate production has believed as adjustment hypoxia to the cancerous cell too. Tumor for its energy requirement

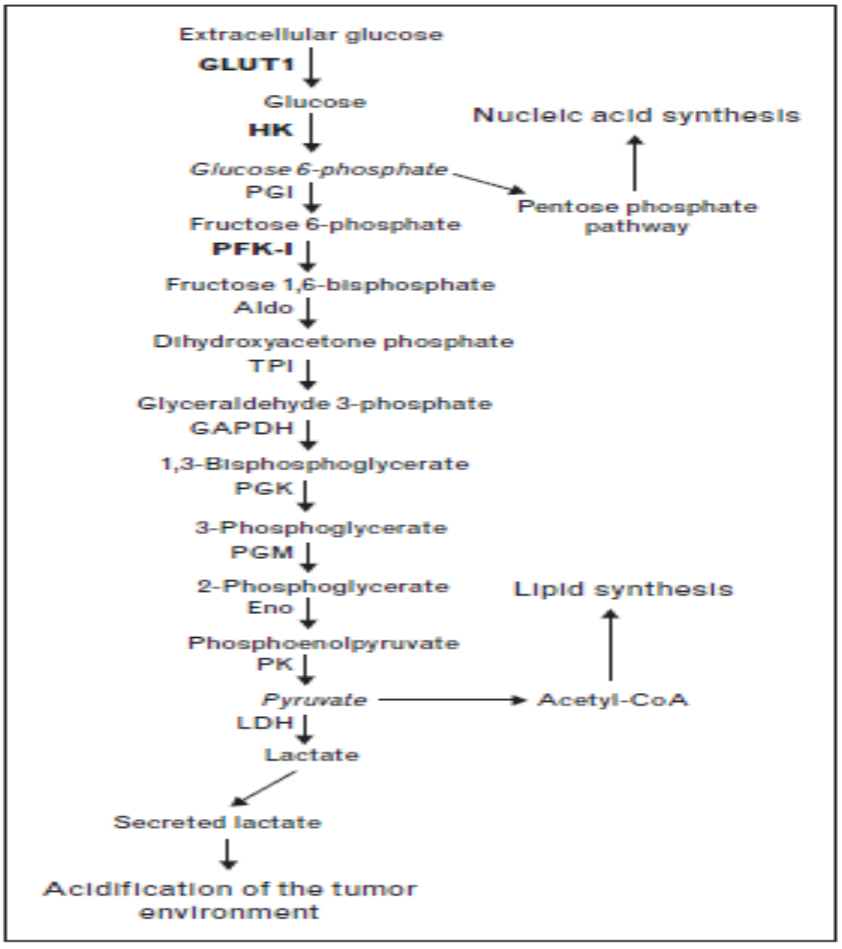


FIGURE 2.4: Glycolysis in Cancer Cells (Annibaldi and Widmann, 2010).

wants to utilize the process of glycolysis even in the condition when the cells are developed in media culture [30].

By the glycolytic pathway, the expanded glucose usage produces intermediates of a metabolic pathway that tumor requires to manage its quick multiplication. Glucose 6-phosphate (G6P) is one of these intermediates utilized for nucleic acid synthesis by the pentose phosphate pathway, to permit quick replication of DNA [48]. Creation of pyruvate inexhaustibly animates synthesis of lipid important for the membranes arrangement in proliferating tumor cells. At last, lactate synthesis of tumor cells initiate tumor microenvironment acidification to make a specific niche to facilitate the development of the tumor and hindering the activity of some anticancer medications [163].

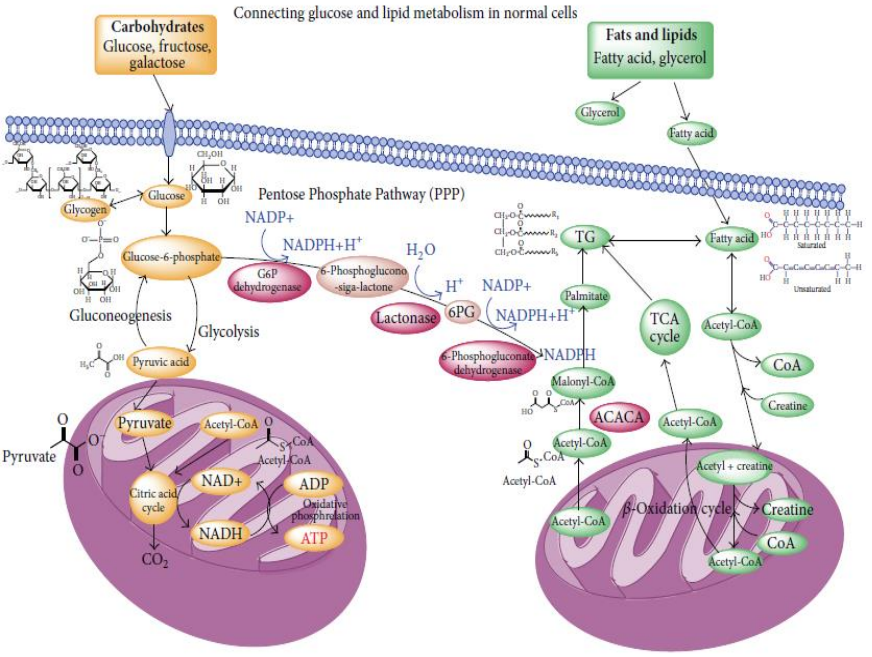


FIGURE 2.5: Metabolism Connection of Glucose and Lipid in normal cells (Beloribi et al., 2016; Cheng et al., 2014; Furuta et al., 2010).

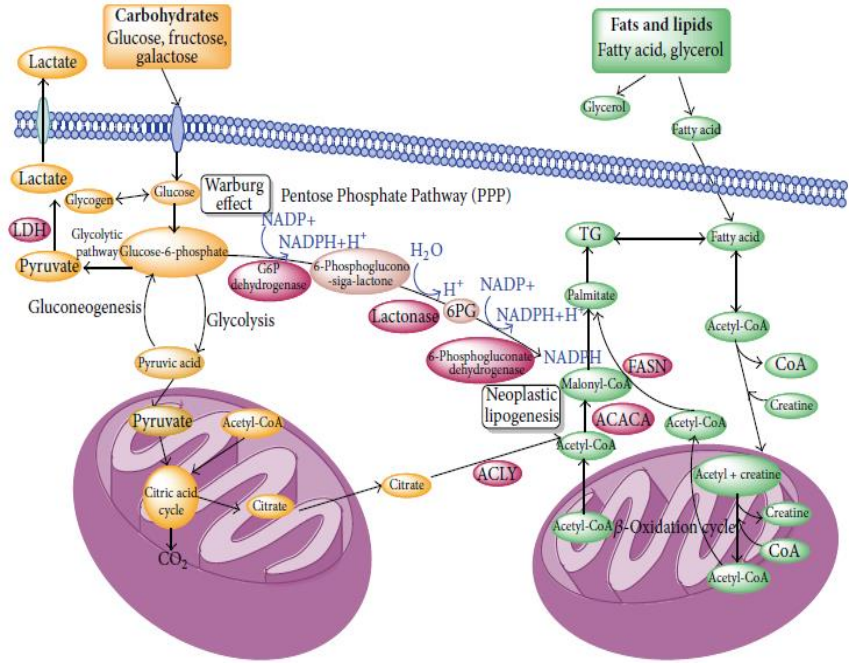


FIGURE 2.6: Fatty Acids synthesis appears to be independent of hormonal regulation in cancer (Beloribi et al., 2016; Cheng et al., 2014; Furuta et al., 2010).

2.7 Lipogenesis and Breast Cancer

Metabolism can enormously vary from individual to individual. These distinctions can have hereditary causes and add to disease chance. They may likewise influence the course of a disease or prompt an antagonistic medication reaction [95]. Deoxycholate, which is integrated by microbes (bacteria) in the intestines, collects in human breast tissue and was found to advance the survival of breast cancer cells at low micromolar concentration [92]. However, initiate apoptosis at higher rates. Breast tumors usually build up a lipogenic phenotype and intensely depend on glucose and glutamine utilization for tumor development. This reinvention of cell metabolism of breast cancer is encouraged by oncogenes and tumor silencer genes and both catalytic [3,31].

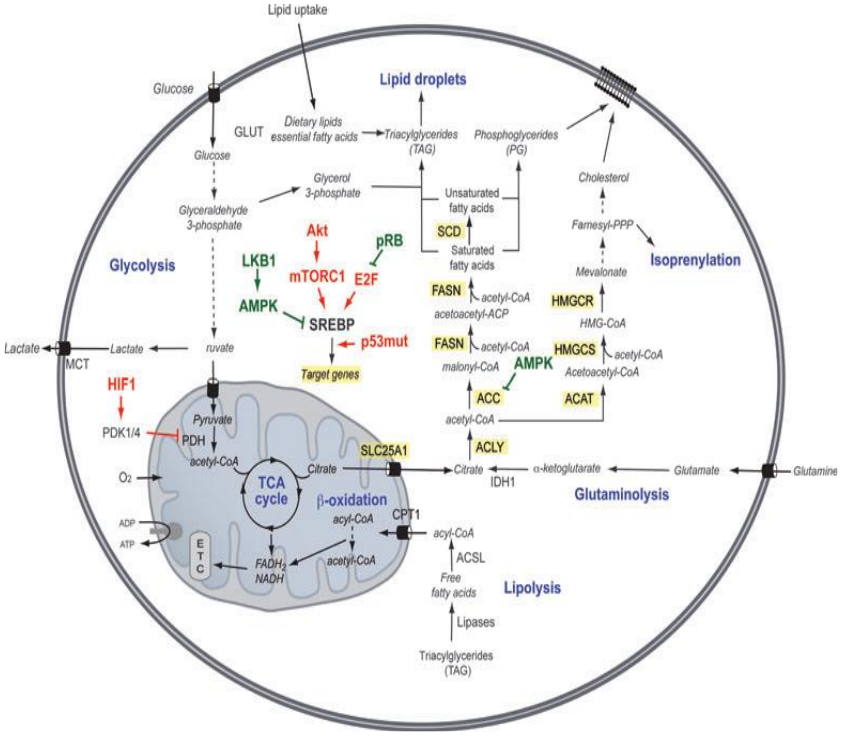


FIGURE 2.7: Lipogenisis pathway (Santos & Schulze, 2012).

Fatty acids synthesis happens in a predetermined number of tissues (i.e., in the lactating mammary organ, fat tissue, and liver) [35,97]. High starch nutrition together with expanded insulin levels in liver and fat tissue fortify fat synthesis to change over abundance sugars into unsaturated fats and triglycerides for energy

storing [69,100]. The formation of palmitate from acetyl-CoA and malonyl-CoA is catalyzed by FASN. Acetyl-CoA-carboxylase is another critical enzyme in fatty acid formation whose work is to feed FASN with malonyl-CoA which is catalyzed to malonyl-CoA from the ATP-subordinate carboxylation of acetyl-CoA, and in this manner going about as the rate-limiting enzymes in the fatty acid formation pathway [103]. SREBP1 is one of the key proteins directing the action of these catalysts and of membrane phospholipid synthesis and this is empowered by insulin which has an important part in hepatocytes [105]. Synthesis pathway in ordinary tissues is managed by nourishment, though in cancer the pathway is dysregulated and out of healthy control. The upregulation of ACC1and FASN is an early occasion in tumor development, In scattered cells of lobules and terminal conduits in ordinary breast tissue the expression of these catalysts (enzymes) happens and in carcinomas the expression ends up noticeably serious and most noteworthy articulation is found in high-review ductal carcinomas in situ [107,109]. In breast cancer, SREBP1 is considered as the key controller of FASN and ACACA. SREBP1 and FASN expression relationship found is not so strong [72], in spite of the fact that it must be remembered that at the post-translational level the function of SREBP1 is managed. MAPK and PI3K pathways managed SREBP1 according to Yang investigations in vitro and that SREBP1 directs transcription of FASN [81]. In Breast tumor cells the key controller is HER2 for ACACA and FASN that are not controlled by SREBP1, and these proteins are directed by the mTOR signaling pathway at the translational level [72]. In this manner [145], the correct part of SREBP1 in managing FASN and ACACA is still in confusion in case of breast cancer. SPOT14 (S14, THRSP) is intensified in 15- 20% of breast cancer, and its expression associated with that of ACC1 and in addition with tumor grade and diminished disease-free survival [70-72]. The activity of SPOT14 is managed by hormones and SREBP1 [55] and the connection between the lipogenic phenotype (i.e., ACC1 and SPOT14 articulation) turned out to be more apparent when the gem structure of SPOT14 was as of late uncovered [113]. Exceedingly proliferative diseased cells demonstrate a solid lipid and cholesterol demand, which they

fulfill by either expanding the take-up of exogenous (or dietary) lipids and lipoproteins or over-activating their endogenous amalgamation (that is, lipogenesis and cholesterol combination, separately [149]. Reprogramming of lipogenic pathway is a standout amongst the most substantial alteration of tumor cell physiology and three genes in this pathway are known to assume key parts in tumor development, specifically ACLY, ACC, and FAS. Important genes engaged in this pathway including ACLY, ACC and FAS are considered to assume basic parts in tumorigenesis and malignancy movement [30]. ACC is more efficient in its active state, in this manner changing over acetyl CoA to malonyl-CoA to synthesize more fatty acids by fatty acid synthase. FAS articulation is related with an increased danger of breast cancer reoccurrence [35] and up control of FAS gives chemoprotection; down direction of FAS causing breast tumor cell line to pretend more sensitive to chemotherapy drugs. Insulin-like growth factor (IGF)-I have been appeared to up direct FAS in malignant breast cancer cells and when FAS was suppressed, IGF-I intervened cell development was hindered [9,86,94].

Target gene expression contemplates recognized up-regulated transcripts associated with the pathway of cholesterol amalgamation and lipogenesis, fundamental for improvement [119] and development of huge forms of cancer cells. Lipogenic catalysts, for example, acetyl-CoA carboxylase (ACC), ATP citrate lyase (ACLY) and fatty acid synthase (FASN) show increased expression level that enhances the formation of cholesterol [178], express all inclusive changes phenotypically in many cancer. Increase level of expression of FASN expects reduced prognosis in cancer patients. The level of expression shows up at the of precancerous stage and continues in prostate and breast tumors [198]. The underlying perceptions, numerous hopeful genes, engaged with cholesterol-related pathways (take-up, union, and capacity) and FAO, believed as essential in supporting threat. The carnitine palmitoyltransferase 1i.e. FAO-constraining enzymes, isoforms C (CPT1A and C) have overexpression in numerous cancers [154]. AMPK and p53 incited up-regulation of CPT1C, incited by, have appeared to shield tumor apoptosis in denied oxygen and glucose levels. Contrarily, CPT1 knockdown sharpens tumor to radiation and causative agents of apoptosis [32].

ACLY bonds metabolic Pathway of glucose and Fatty Acid by the conversion of citrate into oxaloacetate and the precursor for fatty acids synthesis i.e. 2 carbon acetyl-CoA, Reduced ACLY decreases the cells ability of metabolism of glucose into lipid as demonstrated adenocarcinoma cells in human by siRNA [83-84]. With variation in metabolism which harms tumor formation of murine and inhibits cancerous cells in the xenograft tumor formation "ACLY" is silenced by shRNA [83-84]or siRNA [89]or by using chemicals inhibited by "SB-204990" [85]. ACLY has become a hopeful target for treatment, as it has acetyl-CoA as the vital product for numerous molecules as a metabolite in the metabolic pathway and for the acetylation of nucleic acids and of proteins works as a substrate [89]. Therefore, by inhibition of production of ACLY may have significant effect on other pathways of metabolism too.

In TCA cycle, citrate synthase produced citrate and has transferred to the cytosol by means of citrate transporter in mitochondria [97]. Then it was converted into cytosolic acetyl-CoA by using ACLY as an important precursor of fatty acid synthesis. However in case of normal cells ACLY expression has low in amount, but in case of different tumors, it has been expressively up-regulated [104,106,110,107-108]. Noteworthy, active form of ACLY i.e. phosphorylated ACLY has found to be positively interrelated with lungs cancer at clinical level [59-60]. Moreover, inhibiting agents of ACLY such as "siRNA and SB-204990" inhibit synthesis of acetyl-CoA which as a result stops the growth of cells in vivo and in vitro [83,98]. With the blockage of ACLY with siRNA can suppress the Akt signaling which further causes the in vitro loss of tumorigenicity. From these results, it has been clear that ACLY majorly involves in the production of tumor and survival of tumor cells and suggests these compounds as a possible target for clinical usage. Hydroxycitric acid HCA a known ACLY inhibitor which noticeably reduced cholesterol levels, triglycerides and LDL without specious harms in studies at the clinical level. The hydroxycitric acid which is derivative of the subtropical plant, Garcinia gummi-gutta, has been utilized as dietary product and outdated treatment in an Asian country (India), proposing hydroxycitric acid as chemo anticipatory diet supplement [30,112].

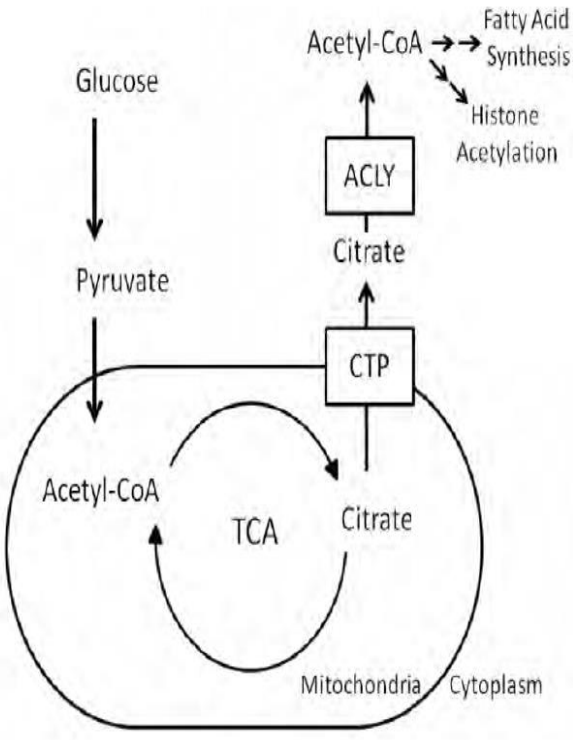


FIGURE 2.8: A model for regulation of Acetyl-CoA with lipogenic genes (Ozkaya et al., 2015).

Acetyl-CoA carboxylase (ACC) form malonyl CoA by carboxylation of acetyl-CoA, catalyzes the dedicated step, and in the FA synthesis pathway is the most regulated enzyme [146]. An ATP dependent enzyme acetyl CoA carboxylase (ACC) carboxylate acetyl-CoA and transforms into malonyl CoA that further act as an active site for enzyme "FAS" in the production of fatty acids [133]. ACC has two isozymes i.e. α and β; their functions have been controlled with the help of different factors such as hormones, nutrition, and another biological stimulus [113]. ACC alpha seems functional for development at an embryonic stage as a mouse deficient with an ACC alpha is embryonic lethal. In contrast, it has been described that in tumor cells levels of ACC alpha of RNA and protein have been increased and these have been also related to the up-regulation of expression of FAS [114]. Remarkably, phosphorylated ACC in lung cancer has initiated to be intensely amplified and other cancer cells, even though it has been found in an unregulated form and its function has been associated with good persistence of diseased (cancer) patients [115].

Palmitate serves as an energy source and an essential cell membrane constituent. However, it serves as a signaling molecule, although in tumor production its role has not clear yet [63]. Nonetheless, a small molecule which can definitely stop the activity of ACC has been observed as predictable anti-cancer drug which will strongly work [30,103].

Fatty Acids Synthase (FAS), with multifunctional ability, consist of seven functional domains (MAT; malonyl-CoA-/acetyl-CoA-ACP-transacylase, KS; β-m ketoacyl synthase, DH; dehydratase, TE; thioesterase, KR; β-ketoacyl reductase, ER; β-enoyl reductase, ACP; acyl carrier protein) [116-118]. All these activities combine use malonyl-CoA and Acetyl-CoA as a donor of carbon and primer respectively for the synthesis of fatty acid. The FAS gene expresses itself in abundance during the development at the embryonic stage, but its expression has delimited to lactating breast, liver, and brain in mature tissues [119-120,115]. Alternatively, in different types of cancers FAS is up-regulated considerably at an embryonic stage and expression of FAS has absolutely related with of patient's poor survival [122-124]. In breast cancer, at the premalignant stage, both HER2 and FAS have expressed such as DCIS (Ductal Carcinoma in Situ) [125-126], and their expression level tends to increase in malignant cells. Significantly, in tumor (cancerous) cells by inhibiting expression of FAS by using small chemicals or siRNA persuades cell arrest and cell death. Therefore, these results suggest that FAS is involved in the early stage of tumor production, feasibly by blocking cell death (apoptosis). FAS gene has been considered a perfect therapeutic target for treatment in cancer cells. Indeed, usage of some pharmacological FAS inhibitors such as C75, cerulenin, and Orlistat can cause the arrest of cell cycle and cell death (apoptosis) for treatment of cancer cells [122-124]. FAS enzyme has a unique secretory form. ELISA, has identified , the rate of expression of FAS in serum has firmly concomitant with the stage of tumor and patients survival in different cancers, suggesting the efficacy of secretion as a prognostic and diagnostic tool [132,135].

2.8 Drugs for FASN

The primary recognized FASN inhibitor is Cerulenin and as a part of anti-infection agents in metabolic pathways [196]. For FASN It is a noncompetitive inhibitor and on the end part of KS domain it binds covalently with the hydroxyl of serine, surrounds hydroxyl-beta-lactam that results in blocking of fatty acid synthesis. the formation of long chain fatty acids is primarily repressed by Cerulenin and leaving the ordinary cells unaffected while other cells restrain the development of cancerous cells [197]. Though, the use of Cerulenin is restricted due to its unstable structure and high poisonous quality levels.

Orlistat (1-(3-hexyl-4-oxooxetan-2-yl) tridecane-2-yl 2-formamido-4-methyl pentanoate) are some Other FASN inhibitors that can possibly be utilized as cancer therapy drugs and advertised as a solution in many countries [198]. Latest studies have demonstrated that the TE domain of the FASN may also be effected by Orlistat. Presently, Orlistat is the main FASN inhibitor under clinical use [199]. The different product from Orlistat, for example, the beta-lactam products of Orlistat distinguished, have indicated the great inhibitory impact on FASN expression. In this manner, for future research on FASN inhibitors, the advancement of Orlistat and its products will be a vital approach [200].

Few natural plant-extracted polyphenols like Epigallocatechin-3-gallate (EGCG) present in green tea is recognized to have a FASN inhibitory impact higher than that of C75 [195]. KR domain of the FASN is affected by EGCG which is considered as a high micromolar time-dependent inhibitor of FASN. According to previous studies, FASN is not only inhibited by EGCG but it also blocks HIF-1-alpha by repressing PI3K/Akt signaling pathway. In recent, EGCG is declared as an inhibitor in tumor development and in breast tumor xenograft models it is used as an important part of study [200-201]. Some limitations are there for EGCG as a FASN inhibitor, and its FASN restraint impact can, in any case, be enhanced. Earlier examinations have demonstrated that, after the solid corrosive and warming treatment of EGCG, FASN inhibition will be significantly expanded, and this raises the chances that the more impact is because of the alteration in

the chemical part of catechin. Though, the subsequent investigation demonstrated that EGCG behaves unstably after strong acid and heat therapy [189-190]. The starting product is unstable too, which makes it hard to examine its molecular structure and properties.

Other FASN inhibitors incorporate urea extracted compounds, for example, GSK83 7149A and can particularly stop the KR domain of FASN [198]. In 2000, Lotfus and his colleagues identified a tiny molecule, a structurally altered type of Cerulenin to be a novel FASN inhibitor, the C75 [196-197]. It is a subsidiary of 3-carboxy-4-alkyl-2-methylenebutyrolactones that reduced the harmfulness of Cerulenin and plays out a superior particular inhibitory impact than Cerulenin. Research has demonstrated that the inhibitory impact of C75 on FASN changed from that of Cerulenin [10,189,198].

Table 2.2: Herbs with promoting blood circulation for removing blood stasis functions with strong FASN inhibitory effect (Cheng et al., 2014).

Promoting blood circulation by removing blood stasis	Chemical component	Pharmacological action
The fruit of Crataegus pinnatifida Bunge	Catabolic acid; Chlorogenic acid; Epicatechin; Epicatechol; Flavonoids	Blocking synthesis of nitrosamine
Rosa chinensis Jacq.	Gallic acid	Anti-breast cancer; antithyroid neoplasm
Paeonia veitchii Lynch	Paeoniflorin; Galloylpaeoniflorin; Paeonol; Lacioflorin; Catechin	Antitumor
Paeonia suffruticosa Andr.	Paeonol; Paeonoside; Paeonolide; Paeoniflorin; Gallic acid; Phytosterol; Alkaloid	Antitumor; immunoregulation; bacteriostat

Spatholobus suberectus Dunn	Daidzein; Epicatechin; Protocatechuic acid; Brassicasterol; Stigmasterol; β-sitosterol; Auriculatin	Antitumor; bacteriostat
Polygonum cuspidatum Sieb, etc Zucc	Polydatin; Emodin; Physcion; Chrysophanol; Citreorsein; Anthraglycside; Resveratrol	Antitumor; immunoregulation; antibiosis; antivirus; elevation of white blood cell counts
Herba lycopi	Essential oils; Flavonoid Glycosides; Saponins; Phenols; Tannins	Antitumor, immunoregulation

Chapter 3

Methodology

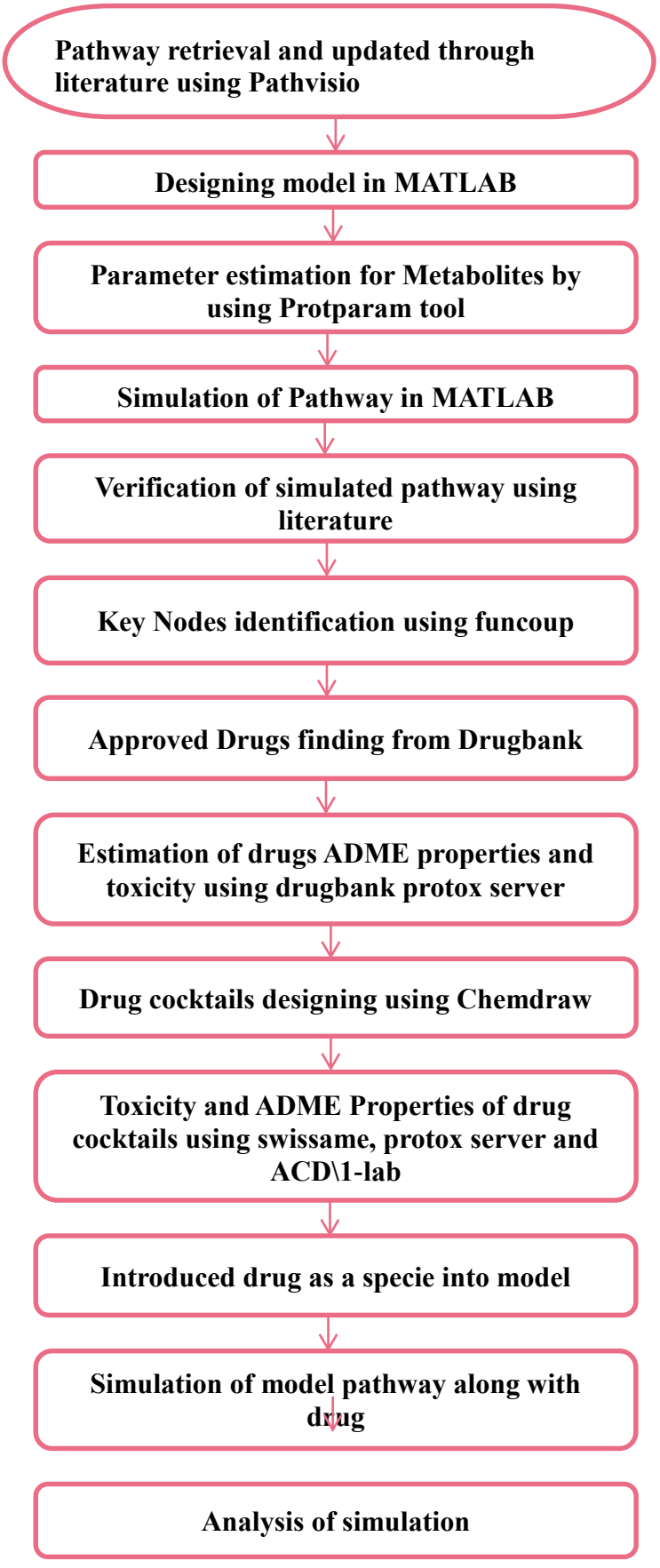


FIGURE 3.1: Flow of the research methodology.

3.1 Pathway Retrieval and Updated through Literature

3.1.1 Glycolysis Pathways

In recent times, analysis based on Protein-Protein Interaction has become an innovative approach or target for drug treatment in cancer and the development of accurate and specific medication [133-134,131-132]. As compared to traditional drug designing method, which mostly emphasizes on the activation or inhibition of a receptor or an enzyme of a single protein that has target for treatment, designing of drug on the bases of PPI which is involved in control of many vital biological procedures by inhibiting or blocking of PPIs interface, it is a very innovative and inventive method for discovery of drug, particularly for treatment of cancer [79]. In many biological studies at the clinical and elementary level, it has been having decided that the hubs and nodes predictions of PPI which have an essential role in transforming cells in case of cancer. And the protein-protein interactions which are related to cancers have turned into therapeutic targets for cancer. With the interference in the reality of protein-protein interaction and with the advancement of technologies for the predictions of modulators in protein-protein interactions and the validation of their pairs, drugs against cancer have made [133,135]. Many targets against protein can find out by using Systems biology which can be repressed instantaneously, as these proteins behave in a network so rather than focusing on a single protein it will provide multi targets [132].

Prominent climaxes of tumor breakdown were found Otto Warburg, demonstrating that growth cells use glucose amount and discharge it as lactate with the availability of oxygen, a mechanism introduced at high-impact known as “glycolysis” or the “Warburg effect” [12]. As compares to it ordinary cells utilizing glucose of mitochondria by means of the tricarboxylic acid in TCA cycle. This sensational increment in glucose take-up by tumor (cancerous cells) has manipulated at clinics

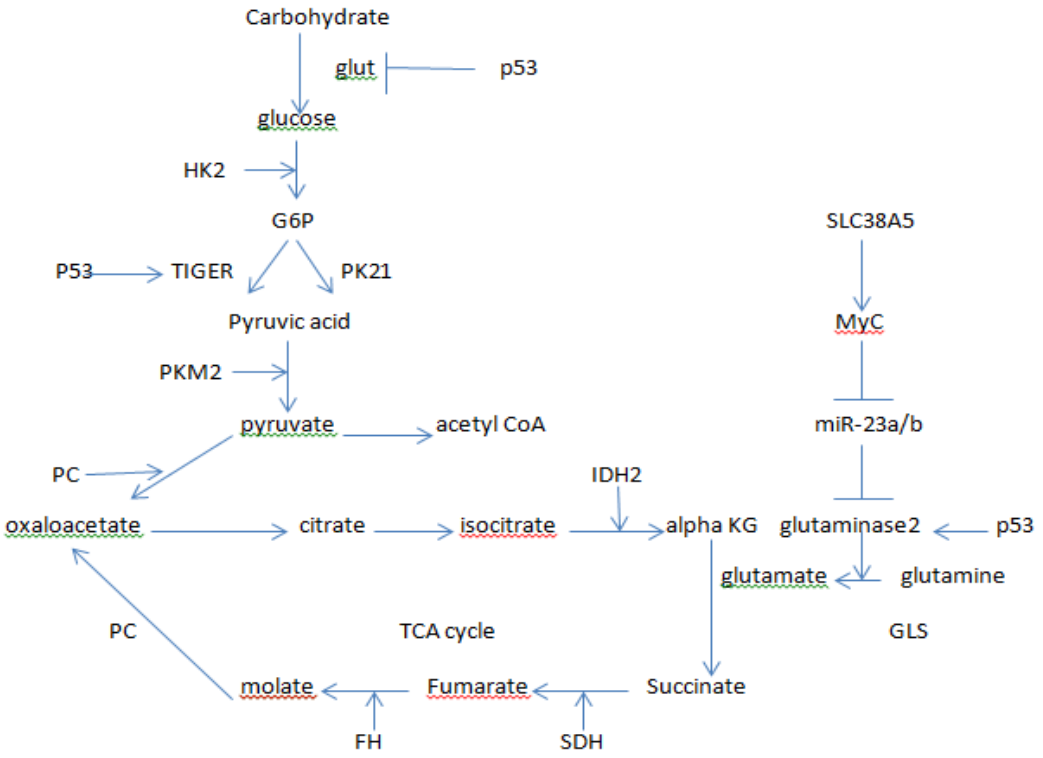


FIGURE 3.2: Metabolism of Carbohydrates.

stage to imagine disease by (18F)- 2-deoxy-D glucose positron outflow tomography (FDG-PET) [172].

The high regulation of pathway is interconnected with the capacity of best growth tumor to metastasize [65]. Moreover, genome studies have revealed serine production is the basis cause for the propagation of many cancers. The genes for the phosphoglycerate dehydrogenase (PHGDH) which is the enzyme for that catalyzes the basic cause to speed up the production of serine, exceptionally communicated in a few tumors, and melanoma and breast disease cells with PHGDH enhancement occupy huge glucose related carbons into glycine and biosynthesis of serine [161,167].

Mutation in IDH has not only reduced the capacity to convert isocitrate to a-ketoglutarate but it also reduces the production of 2 hydroxyglutarates (2HG) by utilizing a-ketoglutarate (143) and that is the numeric condition in AML and glioma. Particularly artificially synthesized inhibitors for the mutation in IDH1 and IDH2 have at clinics trials [152].

The helpful impacts of focusing on a few metabolic proteins have been explored. For example, glycolytic inhibitors, for example, GLUT1inhibitor and 2-deoxyglucose experienced trials in clinics [146,10,45].

TCA cycle derived citrate and NADPH synthesized endogenous fatty acid, which can be delivered by different catalysts and PPP. In the cytosol, ACL converted the citrate into oxaloacetate and acetyl-CoA [62].

3.1.2 Lipogenesis

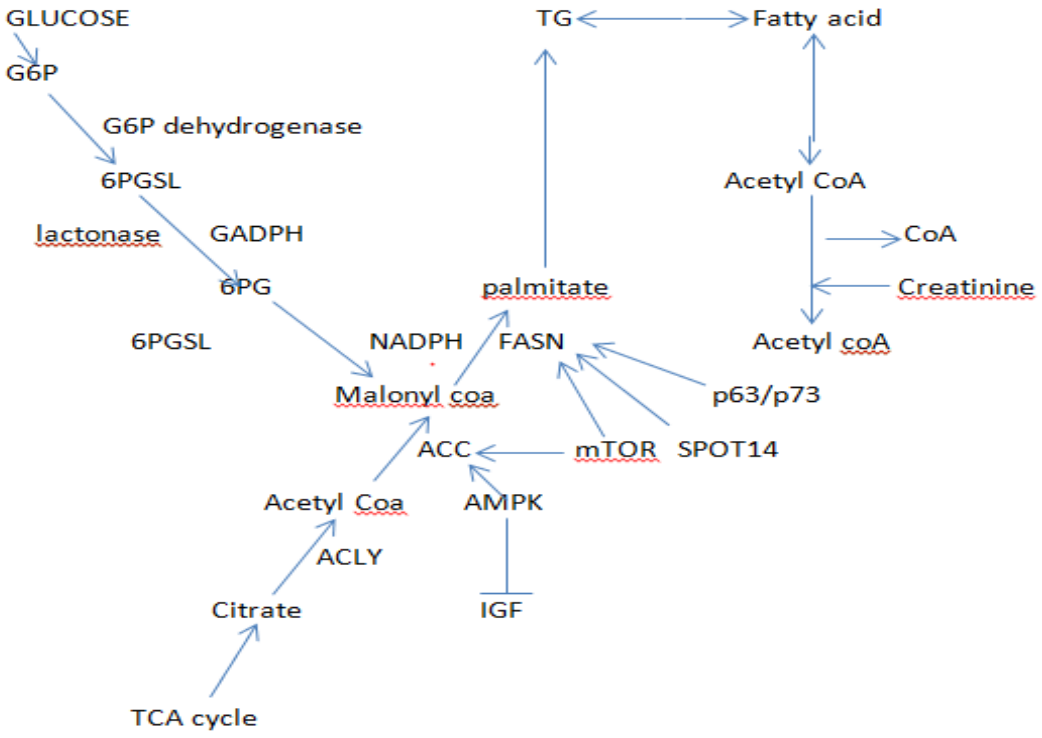


FIGURE 3.3: Lipogenisis Pathway.

High starch nutrition together with increased insulin levels in liver and fat tissue fortifies fat synthesis to change over overabundance sugars into unsaturated fats and triglycerides for energy storing [69,100]. The formation of palmitate from acetyl-CoA and malonyl-CoA is catalyzed by FASN. Acetyl-CoA-carboxylase is another critical enzyme in fatty acid formation whose function is to feed FASN with malonyl-CoA which is catalyzed to malonyl-CoA from the ATP-subordinate carboxylation of acetyl-CoA, and in this manner going about as the rate-limiting

enzymes in the fatty acid formation pathway [103]. SREBP1 is one of the key proteins directing the action of these catalysts and of membrane phospholipid synthesis and this is empowered by insulin which has an important part in hepatocytes [105].

The upregulation of ACC1and FASN is an early occasion in tumor development, In scattered cells of lobules and terminal conduits in ordinary breast tissue the expression of these enzymes happens and in carcinomas the expression ends up noticeably serious and most noteworthy articulation is found in high-review ductal carcinomas in situ [107,109]. In breast cancer, SREBP1 is considered as the key controller of FASN and ACACA.

Lipogenic catalysts, for example, acetyl-CoA carboxylase (ACC), ATP citrate lyase (ACLY) and fatty acid synthase (FASN) show increased expression level that enhances formation of cholesterol [178], express all inclusive changes phenotypically in many cancer. Increase level of expression of FASN expects reduced prognosis in cancer patients. The level of expression shows up at the sore stage of precancerous and continues in prostate and breast tumors [198].

3.1.3 Signaling Pathway

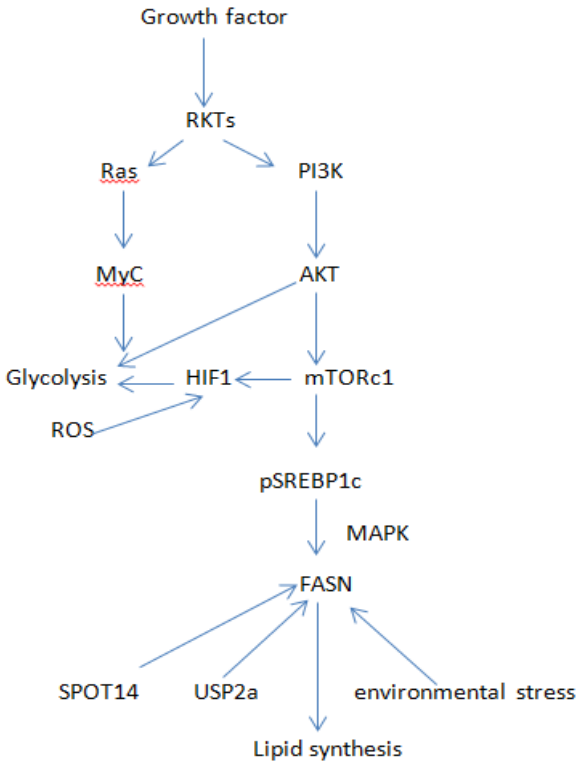


FIGURE 3.4: Signalling Pathway
(DeBerardinis & Chandel, 2016; Cheng et al., 2014; Zhang, 2012).

3.2 Model Development

The model of metabolic Pathways is drawn in MATLAB, is a high-performance language for technical computing. It integrates computation, visualization, and programming in an easy-to-use environment where problems and solutions are expressed in familiar mathematical notation. Typical uses include Data analysis, exploration, and visualization. MATLAB Simbiology, which provides a block diagram editor for building models, or can create models programmatically using the MATLAB language. SimBiology includes a library of common PK models, which you can customize and integrate with mechanistic systems biology models.

3.3 Parameters Estimation

With the help of Protparam tool parameters of all proteins were collected. Parameters mean size or weight represented specifically in units. ProtParam, a tool which certifies the estimation and calculation of different chemicals and physical parameters for a given protein existing in the format of TrEMBL or Swiss-Prot and also facilitates sequences of proteins. The calculated parameter provides the hypothetical PI, atomic composition, molecular weight, amino acid composition, estimation of half-life, aliphatic index, extinction coefficient instability index, hydropathicity grand average [111].

3.4 Simulation of Pathway

A simulation of the system is basically the operation of the system model. Simulations are used to check the system behavior by representing it as a mathematical model. Prior to the model can be simulated it is crucial to determine the underlying values and parameters produced for simulating the model [54][89].

3.5 Drug Cocktail and Parameter Estimation of Drugs

By using the 2D structures of above drugs of FASN and SLC25A1, 5 different combinations of functional groups has made as a "Drug cocktail" which is a modified drug designed with the best combinations of functional groups of FDA approved drugs for breast cancer with the help of Chemdraw, a tool for drawing and editing of molecules developed by David A. Evans and Stewart Rubenstein in 1985 but now sold to PerkinElmer in 2011. It's a really helpful tool in joining of functional groups because it shows error if the bond/linkage is not accurate.

All the parameters i.e. physiochemical properties, ADME properties of drugs are calculated by using "swissadme" a free web tool for evaluating the pharmacokinetics, medical chemistry friendliness of small molecules, drug likeness etc. it describes the physiochemical properties and predict ADME paramters. It is easily accessible at www.swissadme.ch.

Physiochemical properties are confirmed from second tool ACD/I-lab also estimate probabilistic effect of drug cocktails on health, it's a predicting engine. It estimates the physiochemical properties, chemical shifts and ADME toxicities. The browser-based I-Lab software also assesses prediction reliability and includes searchable content databases.

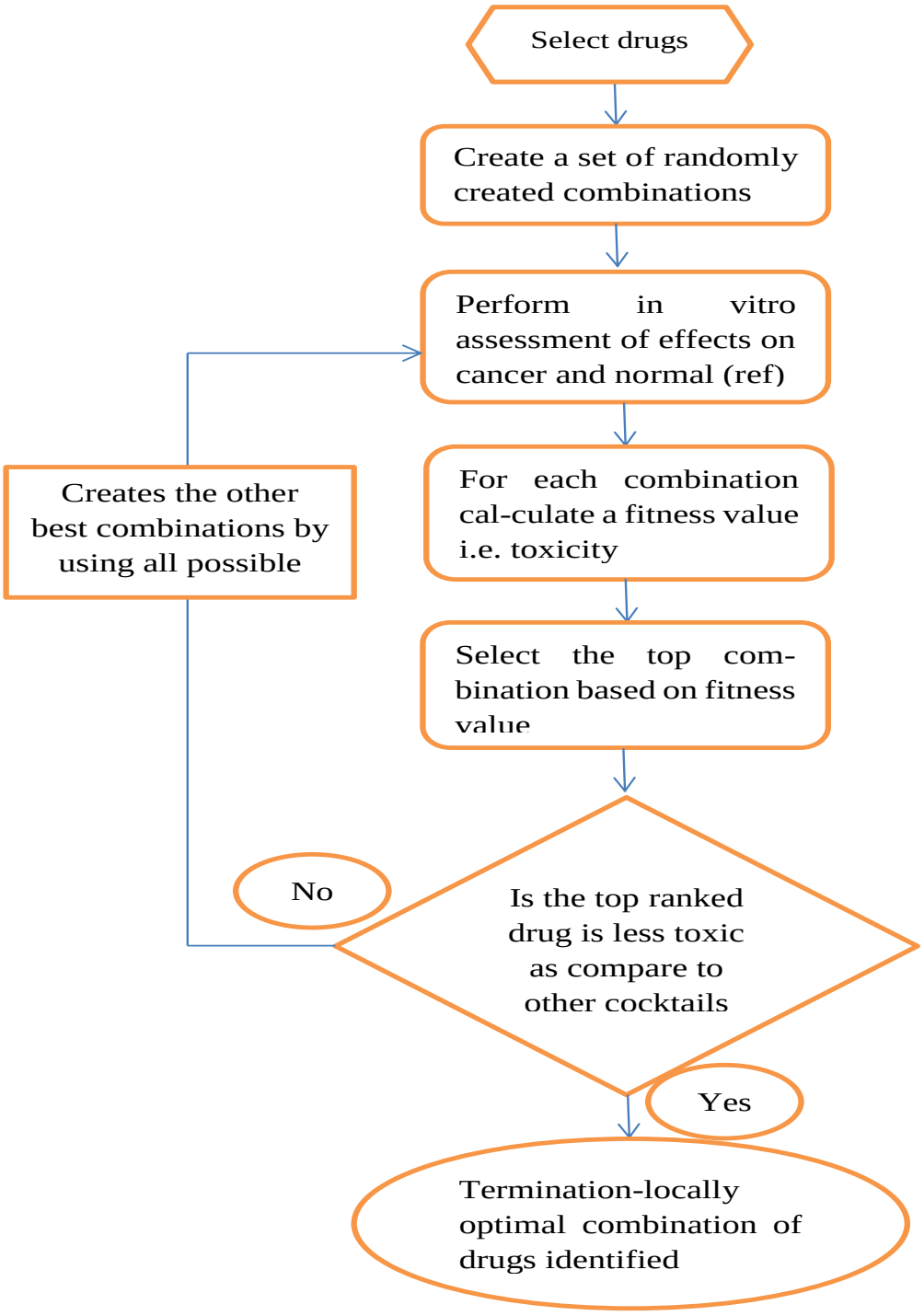


FIGURE 3.5: Strategy for drug cocktail designing.

Chapter 4

Results and Discussion

4.1 Pathway Retrieval and Updated through Literature

4.2 A Comprehensive pathway

The metabolic pathways are closely associated with the Signaling Pathway, such as many of the steps of metabolism of Carbohydrates are controlled by P53 or other cell growth controlling signaling genes and proteins. Similarly, in case of Liopgenisis, many important enzymes are controlled by signaling pathway, for example, SREBP-1 is a gene involved in the production of FASN, PI3k/Akt, HER-2, and mTOR etc are important regulatory elements of metabolic pathways without these elements completion of the process is impossible. That is the reason we combine these three pathways by using a tool "PathVisio", a tool for editing and analyzing the Biological Pathways. It is an open source project founded by Department of Bioinformatics at Maastricht University and Gladstone Institutes. It allows drawing, editing and analyzing biological pathways. It helps in visualizing own experimental data on the pathways and find relevant pathways that are over-represented in your data set. It has its own Plugins that are extensions for

providing advanced analysis method, visualization options and extra functionality of import/export. It can be easily accessed at https://www.pathvisio.org. A comprehensive pathway based on glycolysis, lipogenesis and growth factors was developed to deposit the interaction of signaling to metabolic pathways using literature.Verification of role of each component of pathway has been done using literature and has been provided in table no. 4.1 and 4.2.

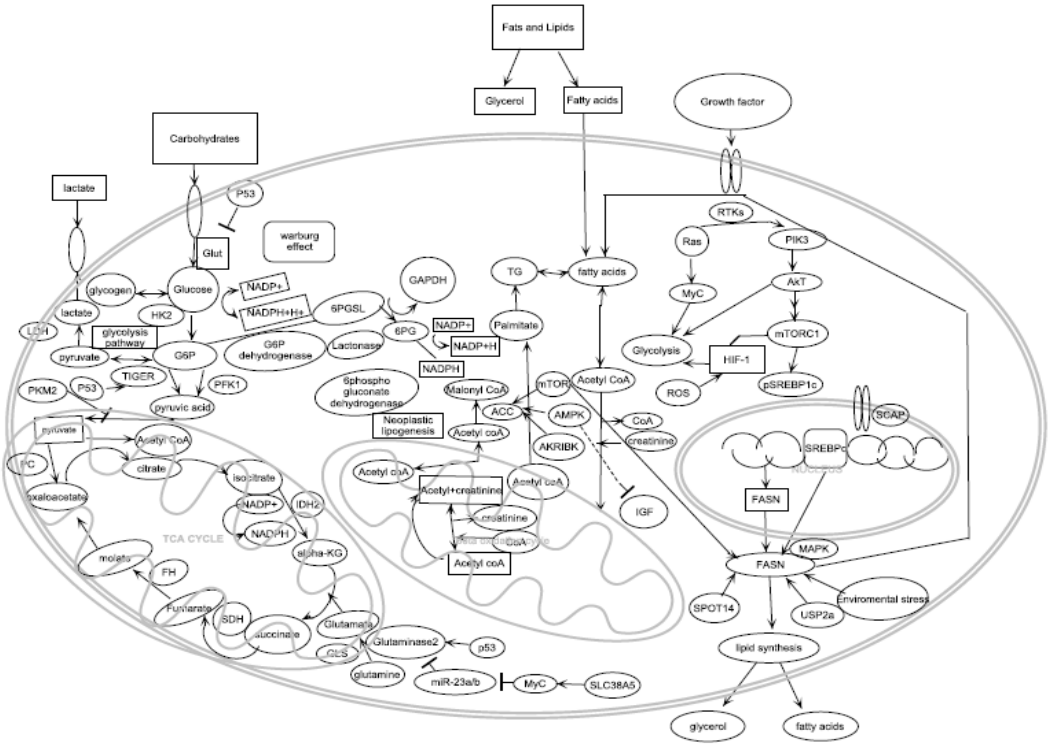


FIGURE 4.1: Combine form of metabolic pathways and Signaling Pathway (https://www.pathvisio.org.)

After a detailed literature study the required pathways of FASN were identified and drawn in the Simbiology toolbox of MATLAB version 2016.

4.3 Parameters Estimation

Reaction rate of each specie in the model required several parameter values such as molecular weight of each gene and or enzyme and kf, shown in the table 4.1.

TABLE 4.1: Verification of glycolysis pathway through literature.

Glycolysis	**References**	**Cell lines**	**Reference**
Carbohydrate→glucose, Glut→p53	[173][10][164] [128]	MCF-7 MDA-MB-436	[10][204][205]
Glucose→G6P, HK2	[173][10][164] [128]	MDA-MB-231 MDA-MB-435	[10][204][205]
G6P→6PGSL, G6P dehydrogenase	[3][10]	MCF-10 MCF-7	[10][204][205]
6PGSL→6PG, lactonase	[3][10]	MCF-10 MDA-MB-436	[10][204][205]
6PG→Malonyl Coa, 6PGDH	[3][10]	MDA-MB-231 MDA-MB-436	[10][204][205]
G6P→Pyruvic acid, TIGAR,PK21	[3][10][103]	MCF-10 MDA-MB-435	[10][204][205]
Pyruvic acid→pyruvate, PKM2	[3][10][103]	SKBR3 MDA-MB-436	[10][204][205]
Pyruvate→acetyl Coa, PDH	[173][164][128]	MCF-7 MDA-MB-435	[10][204][205]
Pyruvate→oxaloacetate, PC	[3][10][103]	MDA-MB-231 MCF-10	[10][204][205]
oxaloacetate→citarte	[3][10][103]	MDA-MB-435	[10][204][205]
Citrate→isocitrate	[3][10][103]	MDA-MB-436	[10][204][205]
Isocitrate→alpha-KG, IDH2	[3][10][103]	MDA-MB-231	[10][204][205]
Alpha-KG→succinate, Glutamate	[128][173]	SKRB3 MCF-7	[10][204][205]
Succinate→Fumarate, SDH	[3][10][164]	MDA-MB-231 MCF-10	[10][204][205]
Fumarate→molate, FH	[3][10][103]	MDA-MB-435 SKRB3	[10][204][205]
Molate→oxaloacetate, PC	[3][10][103]	MCF-7 MDA-MB-436	[10][204][205]

TABLE 4.2: Verification of lipogensis pathway through literature.

Lipogensis	**References**	**Cell line**	**Reference**
Citrate→Acetyl Coa ACLY	[3][10][202][203]	MCF-7 MDA-MB-231	[10][202] [205][204]
AcetyCoa→MalonylCoa ACC	[3][10][164][202][203]	MBA-MB-468 MBA-MB-231	[202][203] [10][205]
MalonylCoa→Palmitate, FASN	[10][202]	MCF-10 MDA-MB-435	[202][203] [10][205]
Palmitate→TG	[10][173][202]	MCF-7 MDA-MB-231	[202][203] [10][205]
TG→Fatty acids	[3][10][164][202]	MCF-10 MDA-MB-231	[202][203] [10][205]
Fatty acid→Acetyl, Coa	[3][10][164][202]	MDA-MB-435 MDA-MB-436	[202][203] [10][205]

Table 4.3: Estimated parameters of Metabolites of pathway (https://web.expasy.org/protparam/)

Metabolite	**No.of A.A**	**Mol. weight**	**Chemical formula**	**Estimated half-life**	**Instability Index**	**Grand-average hydropathicity**	**kf**
Carbohydrate	770	86943.25	$C_{3774}H_{5910}N_{1048}O_{1227}S_{42}$	30 hours	40.69	0.584	0.586
Glucose	492	54083.78	$C_{2503}H_{3916}N_{622}S_{23}$	30 hours	36.57	0.534	0.518
G6P	515	59256.57	$C_{2662}H_{4123}N_{727}O_{765}S_{22}$	30 hours	40.30	0.370	0.354
G6P dehydrogenase	515	59256.57	$C_{2662}H_{4123}N_{727}O_{765}S_{22}$	30 hours	40.30	0.370	0.354
TCA cycle	536	59834.76	$C_{2663}H_{4173}N_{731}O_{800}S_{20}$	30 hours	43.76	0.473	0.457
lactonase	354	39607.49	$C_{1797}H_{2802}N_{458}O_{525}S_{12}$	30 hours	32.86	0.021	0.134
6PG	636	74571.25	$C_{3261}H_{5001}N_{967}O_{959}S_{46}$	30 hours	38.14	0.944	0.928
6PGDH	483	53139.98	$C_{2736}H_{3725}N_{643}O_{694}S_{23}$	30 hours	26.44	0.150	0.134
SLC25A1	311	34012.69	$C_{1529}H_{2447}N_{435}O_{417}S_{13}$	30 hours	21.19	0.065	0.049
Pyruvic acid	1178	129633.5	$C_{5767}H_{9123}N_{1631}O_{1690}S_{48}$	30 hours	39.02	0.169	0.153
Pyruvate	574	61830.17	$C_{2728}H_{4437}N_{787}O_{810}S_{19}$	30 hours	36.91	0.041	0.025
Citrate	1101	120839.2	$C_{5422}H_{8544}N_{1446}O_{1578}S_{50}$	30 hours	33.07	0.105	0.089
Iso-Citrate	414	46659.60	$C_{2085}H_{8544}N_{1446}O_{1578}S_{50}$	30 hours	28.16	0.392	0.023
FASN	2511	273426.6	$C_{12169}H_{19258}N_{3382}O_{3602}S_{89}$	30 hours	45.94	0.070	0.054
Triglycerides	267	30777.83	$C_{1367}H_{2173}N_{381}O_{419}S_{4}$	30 hours	40.83	0.717	0.701
Palmitate	533	60947.55	$C_{2797}H_{4218}N_{708}O_{790}S_{17}$	30 hours	38.00	0.237	0.221
Fatty Acids	472	53953.36	$C_{2409}H_{3764}N_{616}O_{689}S_{21}$	30 hours	37.47	0.015	0.000839
Acetyl-CoA	559	64009.28	$C_{2838}H_{4484}N_{798}O_{837}S_{26}$	30 hours	49.44	0.461	0.445
Malonyl-CoA	493	55003.40	$C_{2467}H_{3912}N_{696}O_{698}S_{16}$	30 hours	40.21	0.204	0.188

4.4 Simulation of Pathway

The designed model was validated for appropriate parameters by performing simulations of the model. Simulations are shown in fig. 4.3.

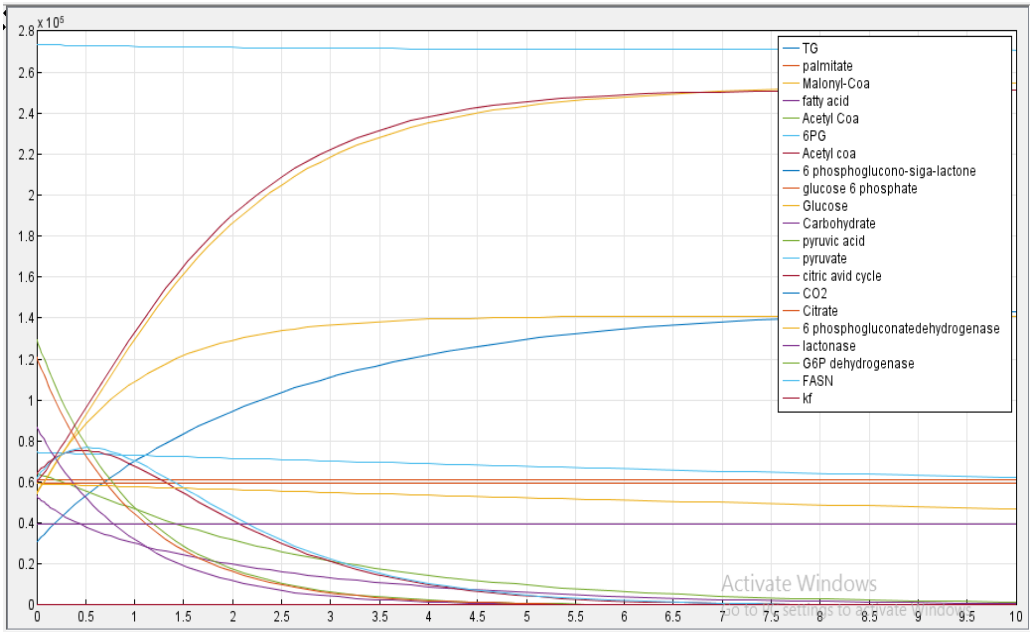


FIGURE 4.2: Simulation of lipogensis pathway.

Figure 4.3 represents the simulations of abnormal pathway in which FASN is over-expressed due to certain mutations, over expression of FASN also results in the down-regulation or up-regulation of other genes of the pathway. The Y axis represents the states that are proteins such as FASN, SLC25A1, Acetyl-CoA, and Malonyl-CoA etc. X axis represents time unit. The simulation time was set for 10 hours and change of rate of regulation of proteins was determined for each ten hours, Different behaviors of proteins were observed in the simulations due to co expression. As the up regulation of acetyl-coA from $0.62e^{-6}$ to $2.5e^{-6}$ also up regulated the malonyl-coA from $0.6e^{-6}$ to $2.5e^{-6}$. Increased amount of acetyl coA is facilitated with the continuous supply of citrate of Citric acid cycle Due to up regulation and down regulation of these proteins, there is a continuous increase in the level of FASN and results in the up-regulation of FASN at $2.73e^{-6}$, which is quite high.

4.5 Identification of Key Nodes for Therapeutic Purpose

The network has been developed by using a framework "fun coup", that infers genome-wide functional coupling in 17 model organisms. The word fun coup refers to the functional coupling. Functional coupling/association is an unspecific form of association that encompasses the direct physical interactions but also more general types of direct or indirect interactions like regulatory interaction or participation of the same pathway. It also differentiates between 5 different classes of interactions. It is easily accessible at http://funcoup.sbc.su.se/search/.

It provides us strongest coupling class for each gene pair and there four hub nodes with black which are responsible for abnormalities in the pathway of lipogenesis. These are FASN, SLC25A1, ACLY, SCD, which have been already verified from the literature to play a key role in Breast cancer. The network of genes is shown in figure 4.4.

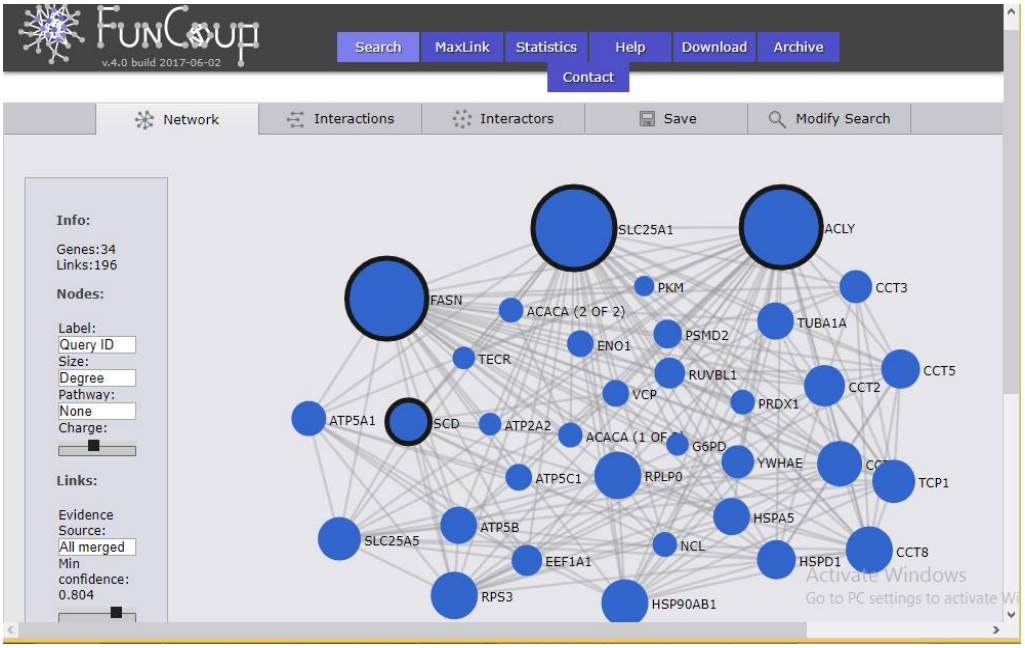


FIGURE 4.3: Hub nodes in lipogensis pathway using FunCoup (http://funcoup.sbc.su.se/search/)

4.5.1 Interactors of Network

The confidence ratio obtained from the network prediction is 80.7. It provides us strongest coupling class for each gene pair and there four-light light genes and proteins with black which are responsible for abnormalities in the pathway of lipogenesis. These are FASN, SLC25A1, ACLY, SCD, which have been already verified from the literature that is playing the key role in Breast cancer. The normal and abnormal functions of these genes and proteins are to regulate the lipogenesis pathway by performing their particular roles i.e. ACLY bonds metabolic Pathway of glucose and Fatty Acid by the conversion of citrate into oxaloacetate and the precursor for fatty acids synthesis i.e. 2 carbon acetyl-CoA, Reduced ACLY decreases the cells ability of metabolism of glucose into lipid as demonstrated adenocarcinoma cells in human by siRNA [83,89]. All these activities combine used malonyl-CoA and Acetyl-CoA as the donor of carbon and primer respectively for the synthesis of fatty acid. The FAS gene expresses itself in abundance during development at the embryonic stage, but its expression has delimited to lactating breast, liver, and brain in mature tissues [114-115,120]. Alternatively, in different types of cancers FAS is up-regulated considerably at an embryonic stage and expression of FAS has absolutely related with of patient's poor survival [122-124].

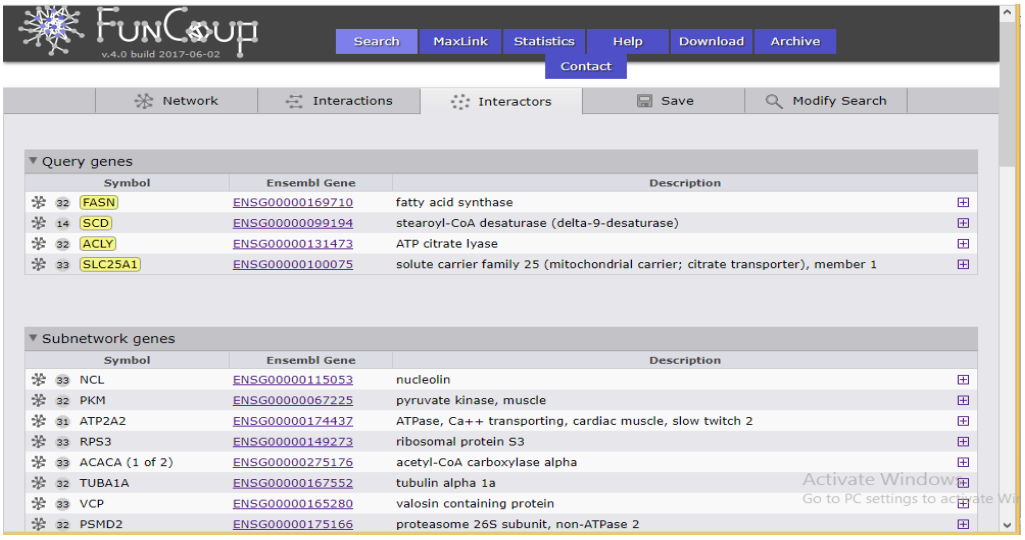


FIGURE 4.4: Representing the interactors of lipogensis pathway (www.funcoup.sbc.)

4.5.2 Interactions of Network

The interaction view lists all the interactions between subnetwork genes and shows detail about how the links that have been derived. Suspected genes are highlighted in Yellow color and Green shows the Orthologs. In log likelihood Ratio, green and red colors show the highly positive and Highly Negative LLR values for different evidence types and species. And the blue color is representing the known coupled pairs in the PPI of our pathway.

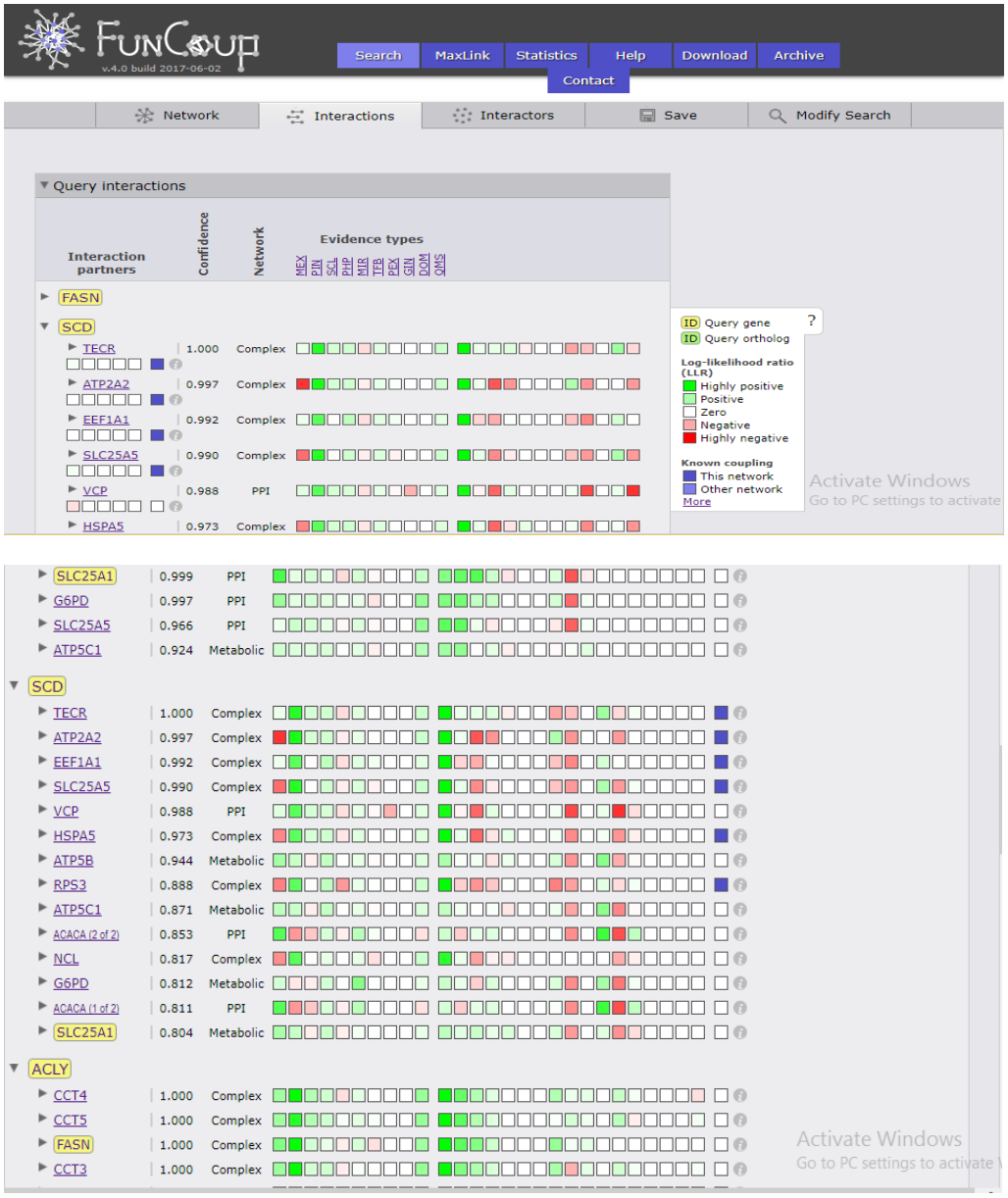


FIGURE 4.5: Representing the interactions between genes of lipogensis pathway (www.funcoup.sbc.)

4.6 Drugs for Key Nodes of Lipogenesis Pathway

All drugs for these highlighted proteins and gene have been obtained from Drug Bank. Durg Bank (www.drugbank.ca) is an extravagantly developed skill that associates certain drug data with an extensive target of drug and metabolite data. Drug Bank has been mostly used to encourage the disclosure of in silico drug targets, drug outline, screening or docking of the drug, prediction of drug metabolism, prediction of drug interaction, and general instruction about pharmaceutics. It has data about FDA-affirmed drug molecules and biotech drugs. Drug Bank can be accessed by using http://www.drugbank.ca(Wishart et al., 2007).

TABLE 4.4: Drugs against FASN (www.drugbank.ca)

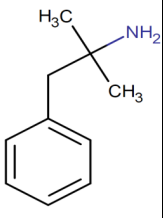
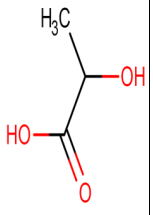
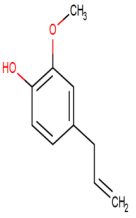
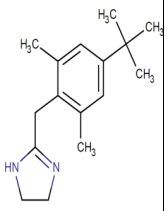
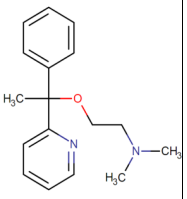
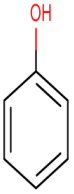
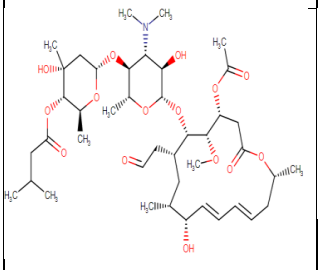
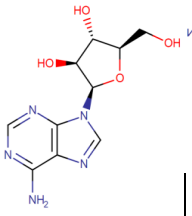
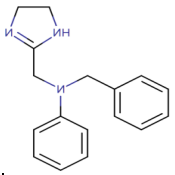
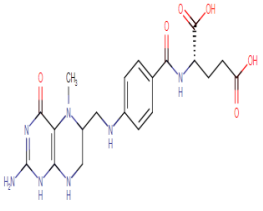
Phentermine	Lactic acid	Eugenol	xylometazoline	Doxylamine	Phenol
					
www.drugbank.ca	www.drugbank.ca	www.drugbank.ca	www.drugbank.ca	www.drugbank.ca	www.drugbank .ca

TABLE 4.5: Drugs against SLC25A1 (www.drugbank.ca).

Josamycin	Vidarabine	Antazoline	5-methyltetrahydrofolic acid
			
www.drugbank.ca	www.drugbank.ca	www.drugbank.ca	www.drugbank.ca

4.6.1 Drug Parameters

All drugs have been collected from Drug bank which has FDA approved drugs and their all physiochemical and ADME properties were estimated by using Swissasme tool (www.swissadme.ch) by directing using structure of cocktails as input. These properties of cocktails and probabilities of their health effects were predicted using ACD/I-lab reports (https://ilab.acdlabs.com/iLab2/).

TABLE 4.6: Drugs against FASN and its Physiochemical and ADME properties (www.drugbank.ca)(www.swissadme)(https://omictools.com/protox-tool)

Drugs FASN	Dosage	Route	Ingredients	Chemical formula	IUPAC name	toxicity	LD50 Value	Status	Type
Phentermine	Capsule /tablet	oral	Phentermine Hydrochloride (3.75 mg/1) + Topiramate (23 mg/1)	$C_{10}H_{15}N$	2-methyl-1-phenylpropan-2-amine	Class 2	10 mg/kg	approved	Small Molecule
Lactic acid	Injection	Intravenous	Sodium lactate (310 mg) + Calcium Chloride (20 mg) + Potassium Chloride (328 mg) + Sodium Chloride (600 mg)	$C_3H_6O_3$	2-hydroxypropanoic acid	Class 3	75 mg/kg	approved	Small Molecule
Eugenol	Liquid/ gel	Topical	Eugenol (.0416 g/g) + Guaiacol (.0416 g/g)	$C_{10}H_{12}O_2$	2-methoxy-4-(prop-2-en-1-yl)phenol	Class 4	1930 mg/kg	Approved	Small Molecule
	spray	Nasal	Xylometazoline hydrochloride (.05 %) + Antazoline sulfate (.5 %)	$C_{16}H_{24}N_2$	2-[(4-tert-butyl-2,6-dimethylphenyl)methyl]-4,5-dihydro-1H-imidazole	Class 3	75 mg/kg	approved	Small Molecule
Doxylamine	Tablet	Oral	Doxylamine succinate(6.25 mg/1) + Acetaminophen (325 mg/1) + Dextromethorphan hydrobromide (15 mg/1)	$C_{17}H_{22}N_2O$	dimethyl({2-[1-phenyl-1-(pyridin-2-yl)ethoxy]ethyl})amine	Class 4	470 mg/kg	Approved	Small Molecule
Phenol	Injection/ solution	Intradermal; Subcutaneous	Phenol (0.45 %) + Benzocaine (6.5 %) + Camphor (0.25 %) + Menthol (0.25 %)	C_6H_6O	Phenol	Class 3	270 mg/kg	Approved	Biotech

TABLE 4.7: Drugs against SLC25A1 and its Physiochemical and ADME properties (www.drugbank.ca)(www.swissadme)(https://omictools.com/protox-tool)

Drugs SLC25A1	Dosage	Route	Ingredients	Chemical formula	IUPAC name	toxicity	Status	Type	LD50 value
Vidarabine	liquid	oral	Not available	$C_{10}H_{13}N_5O_4$	(2R,3S,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol	Class 2	Approved, Investigational	Small Molecule	8mg/kg
Antazoline	Capsule/ liquid drops	Ophthalmic/oral	Antazoline (225 mg/1) + Cholecalciferol (6.25 ug/1) + Cyanocobalamin (15 ug/1) + Folic Acid(1 mg/1) + Iron (38 mg/1) + Magnesium oxide(15 mg/1) + Pyridoxine hydrochloride (30 mg/1)+ Tocopherol (10 mg/1) + Calcium ascorbate (18 mg/1) + Zinc oxide (1 mg/1)	$C_{17}H_{19}N_3$	N-benzyl-N-(4,5-dihydro-1H-imidazol-2-ylmethyl)aniline	Class 4	Approved	Small Molecule	398mg/kg

5-methyltetrahydrofolic acid	Tablet/ capsule	Oral	5-methyltetrahydrofolic acid (.5 mg/1) + Sodium sulfate (8 mg/1) + Sulfur (388 mg/1)+ Thiosulfuric acid (1.8 mg/1)	$C_{20}H_{25}N_7O_6$	(2S)-2-[(4-{[(2-amino-5-methyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl)methyl]amino}phenyl)formamido]pentanedioic acid	Class 4	Approved, Nutraceutical	Small Molecule	1000mg /kg
Hydroxypropyl cellulose	Pellet	Ophthalmic	**Lacrisert** 5 mg/1				Approved	Small Molecule	10200 mg/kg (oral, rat)
Josamycin	-			$C_{42}H_{69}NO_{15}$	(2S,3S,4R,6S)-6-{[(2R,3S,4R,5R,6S)-6-{[(4R,5S,6S,7R,9R,10R,11E,13E,16R)-4-(acetyloxy)-10-hydroxy-5-methoxy-9,16-dimethyl-2-oxo-7-(2-oxoethyl)-1-oxacyclohexadeca-11,13-dien-6-yl]oxy}-4-(dimethylamino)-5-hydroxy-2-methyloxan-3-yl]oxy}-4-hydroxy-2,4-dimethyloxan-3-yl 3-methylbutanoate	Class 4	Approved, Investigational	Small Molecule	1000mg /kg
Interferon Alfa-2a, Recombinant	Liquid/ Powder for solution	Intramuscular; Subcutaneous		$C_{860}H_{1353}N_{227}O_{255}S_9$			Approved, Investigational	Biotech	

4.6.2 Drug Cocktails

In the field of therapeutics "Systems biology" recreates the regulatory interactions and relationships within metabolic, genetic, and PPI networks. These networks of biological pathways are highly complex consequently healthiness and specifity are their important features [64-65]. Due to interweaving of networks that show with the inhibition of single protein. It is not possible to suppress the expression of whole network [179-180]. So with the help of "Systems biology" researcher has become able to target many proteins by using drugs with their modified forms as combinations of drugs titled as drug cocktail, which have less toxicity and show more stable results in the behavior of any abnormal/mutated network . And at the same time this strategy will provide the more beneficial effects. Furthermore recently in the field of research it has validated that inhibition of PPI has become a different innovative strategy for treatment of cancer [98].

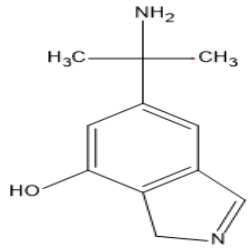
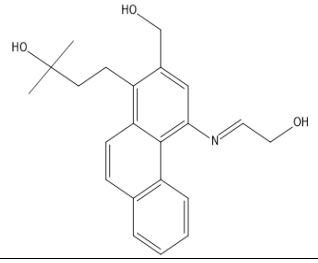
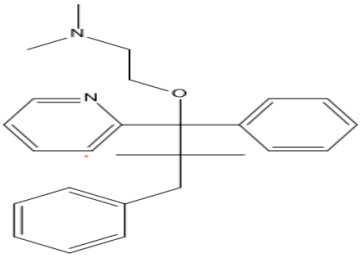
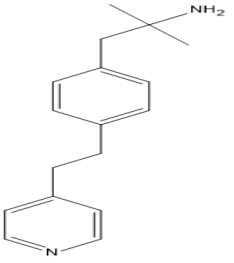
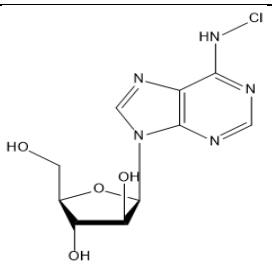
All the below given information about physiochemical and ADME properties have been calculated by using "Swissadme" at www.swissadme.

Health effects and probabilistic effects of all cocktails on the health have been estimated by using ACD/I-lab.

4.6.3 Cocktails Design

About five different drug cocktails were designed by combining the active functional groups of the drugs, already used to treat FASN and other key nodes. The designed cocktails are shown in table 4.7.

TABLE 4.8: Structure of Drug Cocktails designed by using Chemdraw

Cocktail 1	
Cocktail 2	
Cocktail 3	
Cocktail 4	
Cocktail 5	

The physiochemical and ADME properties of the cocktails are shown in table 4.8.

TABLE 4.9: The physiochemical and ADME properties of the cocktails (www.drugbank.ca)(www.swissadme)(https://omictools.com/protox-tool)

	Cocktail 1	**Cocktail 2**	**Cocktail 3**	**Cocktail 4**	**Cocktail 5**
Chemical formula	C11H14N2O	C22H25NO3	C26H32N2O	C17H22N2	C10H12ClN5O4
Toxicity	Class 4	Class 5	Class 3	Class 2	Class 2
LD50	800 mg/kg	2500 mg/kg	470 mg/kg	35 mg/kg	13 mg/kg
Molecular weight	190.24 g/mol	351.44 g/mol	388.55 g/mol	254.37 g/mol	301.69
LogBB	0.01	0.01	0.24	0.14	0.01
TPSA	58.61	73.05	25.36	38.91	125.5
LogS	-1.35	-3.96	-5.52	NA	-1.69
Logp	1.76	3.23	4.10	2.98	1.18
Density	1.22	1.16	1.052	1.035	2.07
No. of H donor	3	3	0	1	4
No. of H acceptor	3	4	3	2	7
Molar Refractivity	54.12	102.06	120.24	80.63	67.56
Molar Volume	155.0	301.2	369.3	245.7	145.4
Lipinski violation	No	No	No	No	No

All the cocktails were also checked for the side effects and several probability values of the side effects were calculated, which determine the ratio of effects of the cocktail on the living organism. The side effects are shown in table 4.9. given below.

Table 4.10: The probabilities of health effects of the cocktails on the organism (https://ilab.acdlabs.com/iLab2/)

Cocktail	**Probability effect on Blood**	**Prob. effect on Cardiovascular**	**Prob. effect on Gastrointestine**	**Prob. effect on Liver**	**Prob. effect on Lungs**	**Prob. effect on Kidney**
Cocktail 1	0.27	0.47	0.52	0.14	0.58	0.06
Cocktail 2	0.93	0.80	0.89	0.64	0.95	0.59
Cocktail 3	0.32	0.79	0.85	0.33	0.88	0.77
Cocktail 4	0.73	0.83	0.70	0.17	0.86	0.6
Cocktail 5	0.58	0.63	0.83	0.81	0.91	0.28

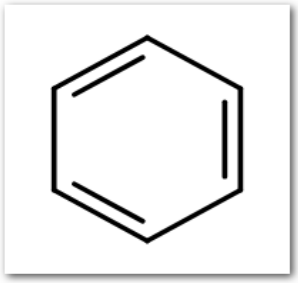
From the results of table 4.6.3 it is confirmed that all the cocktails have very minimal side effects and none of the probabilities is greater than 1.0.

TABLE 4.11: Toxicity of Cocktails (https://omictools.com/protox-tool)

Cocktail	**Cocktail 1**	**Cocktail 2**	**Cocktail 3**	**Cocktail 4**	**Cocktail 5**
Toxicity	Class 4	Class 5	Class 3	Class 2	Class 2

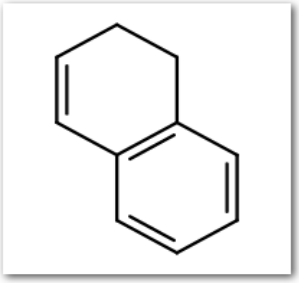
As cocktail 2 is less toxic as compare to other cocktail that is the reason of it's selection and introduced as dose in the abnormal pathway modeled in Simbiology MATLAB (2016).

Functional Groups of Cocktail 2 which is used in Drug controlled Simulation.



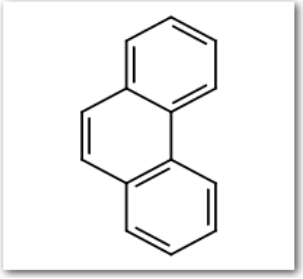
Pa	Pi	Side Effect
0.483	0.205	Hepatotoxicity
0.414	0.174	Arrhythmia
0.396	0.072	Myocardial infarction
0.382	0.113	Cardiac failure

FIGURE 4.6: Aromatic benzene (a)



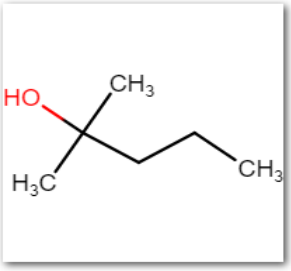
Pa	Pi	Side Effect
0.436	0.159	Arrhythmia
0.354	0.109	Myocardial infarction

FIGURE 4.7: Benzene with fig (a)



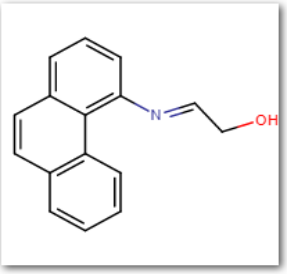
Pa	Pi	Side Effect
0.584	0.031	Myocardial infarction
0.496	0.198	Hepatotoxicity

FIGURE 4.8: Aromatic benzene with fig (b)



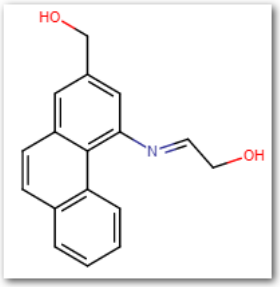
Pa	Pi	Side Effect
0.647	0.121	Hepatotoxicity
0.241	0.240	Nephrotoxicity

FIGURE 4.9: Hydroxy methyl group (c)



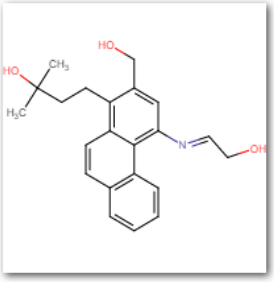
Pa	Pi	Side Effect
0.466	0.214	Hepatotoxicity
0.390	0.075	Myocardial infarction
0.363	0.123	Cardiac failure

FIGURE 4.10: fig (b) with (c), (e)



Pa	Pi	Side Effect
0.504	0.043	Myocardial infarction
0.335	0.316	Hepatotoxicity

FIGURE 4.11: fig (e) wih hydroxyl group, (f)



Pa	Pi	Side Effect
0.667	0.113	Hepatotoxicity

FIGURE 4.12: fig (f) with (c), (g)

Pa (probability "to be active") provide the probability that the compound is fit in to the sub-class of active compounds

Pi (probability "to be inactive") provide the probability that the compound is fit in to the sub-class of inactive compounds

Only activities with Pa > Pi are considered as possible for a particular compound.

If The Pa ¿ pi and is less than 1.0, it means there are very few chances that it can produce predicted side effects, there are 90% chances that the compound has a high novelty and may become New Chemical Entity (NCE), if the pa > 1.0 it means that there are 90% chances that it can produce the predicted side effects.

4.7 Simulation of Pathway with Dose

4.7.1 Model Development

The developed pathway model is given in figure 4.13.

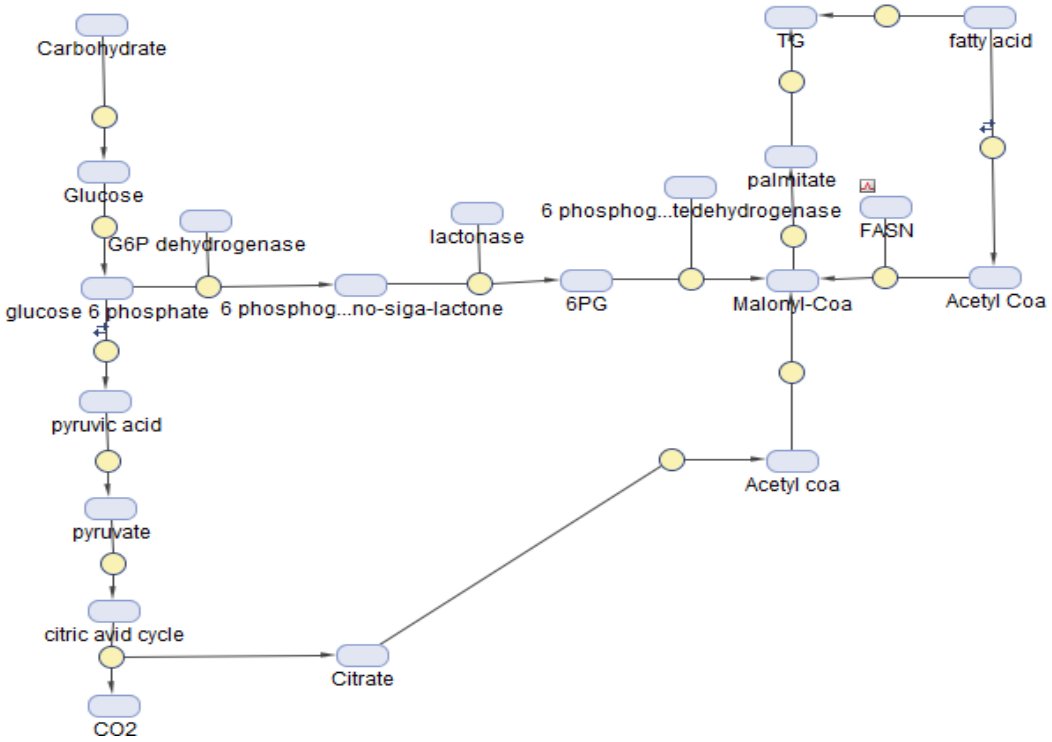


FIGURE 4.13: Model development of metabolic pathway.

In the metabolism, normal cells utilized the glucose of mitochondria by means of tricarboxlic acid in TCA cycle. The glucose is converted into pyruvate with the help of important enzymes and introduced into TCA cycle for the production of energy but in case of aggressive cancer cells which not only relay on glycolysis

i.e Warburg Effect, also interact with lipid metabolism [10]. It starts a dietary independent method, utilizes high rate of de novo fatty acid synthesis. In which more production of acetyl-coA and malonyl-coA starts which further catalyzed by FASN to produce palimtate and triglycerides/16-C saturated fatty acids. In the pathway each gene and or enzyme is linked with a certain reaction the $\leftrightarrows$ sign in the model represents the reversible reaction,$\rightarrow$ represents the forward reaction from one metabolite to other. The FASN is of main concern in this research work therefore FASN is represented as being dosed by cocktail in the model.

4.7.2 Dose with 100mg Drug

100 mg dose of cocktail was induced in the model and FASN was set as the drug target to determine the efficacy of cocktail 2. Following simulations were produced

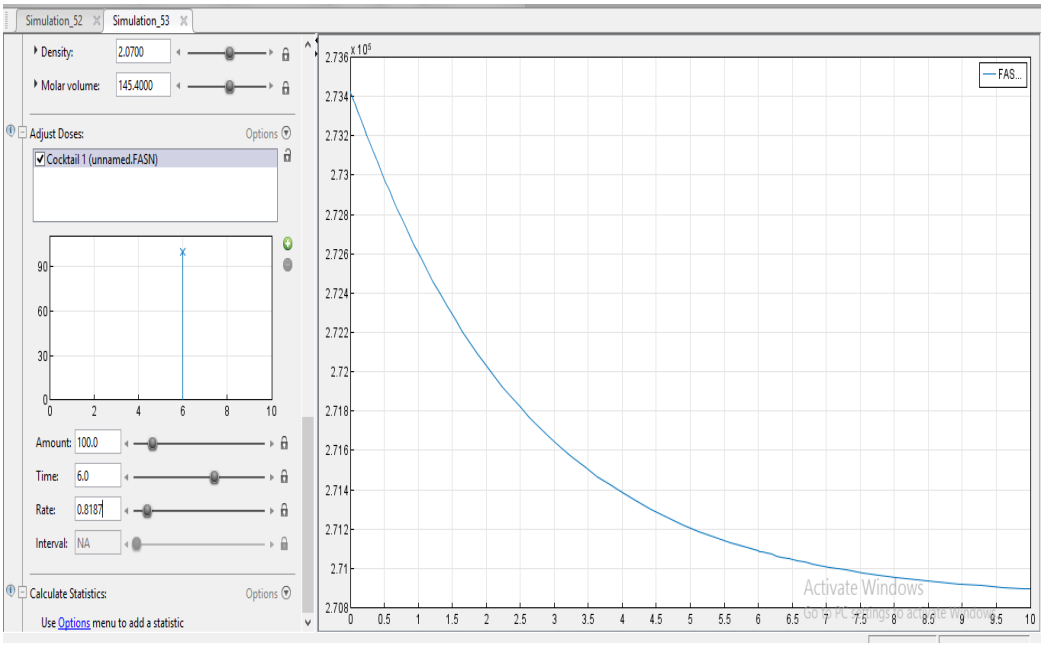


FIGURE 4.14: Simulations for 100 mg dose of cocktail 2.

Y-axis representing the level of FASN and X-axis represents the dose time. From the above graph it is clear that first the level of FASN was more than $2.74e^{-6}$, but when the 100 mg cocktail is induced the FASN start to decrease from $2.73e^{-6}$ to $2.70e^{-6}$. If patient is suffering from breast cancer due to overexpression of

FAS in lipogensis which is strongly responsible for more de novo lipogensis w.r.t compensating energy level for the cancerous cells, intake the given drug cocktail continuously then the level of FAS will gradually be down-regulated.

4.7.3 Dose with 170mg Drug

Similarly several other dose values were also induced in the model to identify the change in the level of FASN

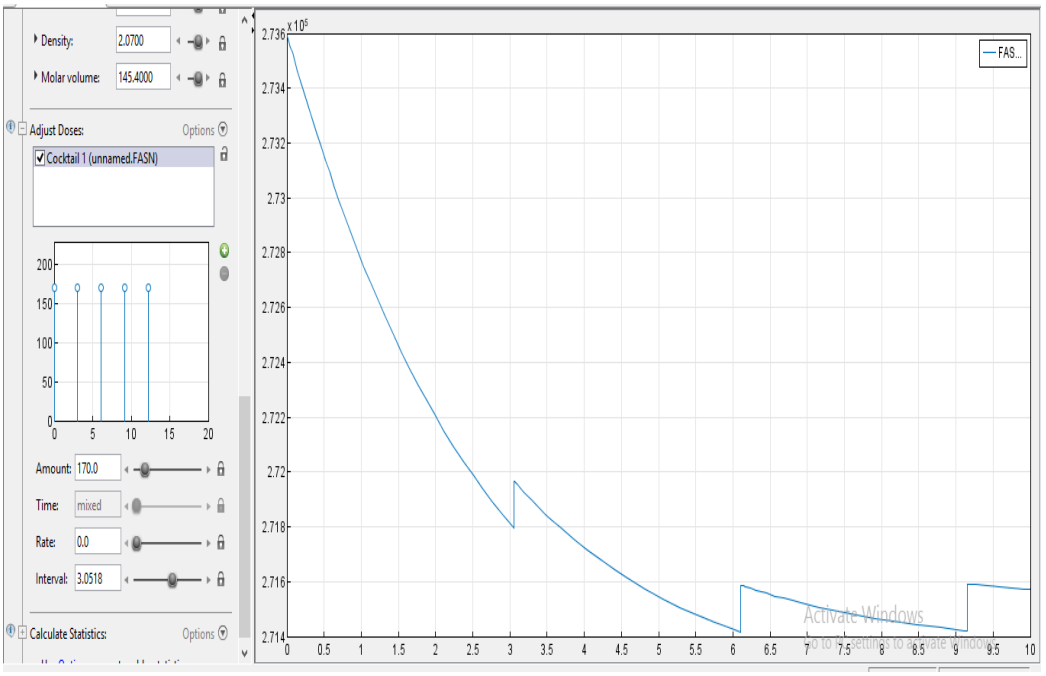


FIGURE 4.15: simulations of FASN for repeated dose.

In the second simulation the same drug cocktail is applied with FAS with increase dose of 170 mg and at that stage it applied with interval of 3 days. Above graph clearly represents that with intake of drug cocktail the FAS level decreases at the same rate from $2.73e^{-6}$ to $2.718e^{-6}$ but after three days when the dose intake stops then level of FAS starts increasing gradually from $2.718e^{-6}$ to $2.72e^{-6}$. Then again with the intake of drug cocktail, FAS is down regulated from $2.72e^{-6}$ to $2.714e^{-6}$. At the end of 2nd interval when dose ends again FAS again is regulated from $2.714e^{-6}$ to $2.716e^{-6}$, with the start of 3rd interval along with dose FAS again shows down regulation from $2.716e^{-6}$ to $2.70e^{-6}$. From the above discussion

it is clear that for the controlled FAS level patient has to intake drug cocktail regularly for its proper down regulation otherwise it will start up-regulating for energy homeostasis of cancer cells.

FAS articulation is related with an increased danger of breast cancer reoccurrence [35] and up control of FAS gives chemoprotection; down direction of FAS causing breast tumor cell line to pretend more sensitive to chemotherapy drugs. Insulin-like growth factor (IGF)-I have been appeared to up-regulate FAS in malignant breast cancer cells and when FAS was suppressed, IGF-I intervened cell development was hindered [9,86,94].

The rate of lipogenesis is additionally essentially elevated in tumor cells with a specific end goal to make up for the higher rate of expansion. It is progressively obvious that numerous genes engaged in metabolic pathways assume coordinate parts in tumorigenesis and tumor development [29].

The formation of palmitate from acetyl-CoA and malonyl-CoA is catalyzed by FASN. Acetyl-CoA-carboxylase is another critical enzyme in fatty acid formation whose work is to feed FASN with malonyl-CoA which is catalyzed to malonyl-CoA from the ATP-subordinate carboxylation of acetyl-CoA, and in this manner going about as the rate-limiting enzymes in the fatty acid formation pathway [103].

Target gene expression contemplates recognized up regulated transcripts associated with pathway of cholesterol amalgamation and lipogenesis, fundamental for improvement and development of huge forms of cancer cells. Lipogenic catalysts, for example, acetyl-CoA carboxylase (ACC), ATP citrate lyase (ACLY) and fatty acid synthase (FASN) show increased expression level that enhances the formation of cholesterol; express all inclusive changes phenotypically in many cancer. Increased level of expression of FASN expects reduced prognosis in cancer patients. The level of expression shows up at the sore stage of precancerous and continues in prostate and breast tumors.

4.8 Differential Equations of Lipogensis Pathways

Several differential equations were developed for the model shown below.

α = 1/Lipogenesis, β = Reaction

d(TG)/dt =α*(β_1.kf*TG + β_5.kf*[fatty acid]-β_5.kr*TG)

d(palmitate)/dt = α*(-β_1.kf*TG+β_2.kf*[Malonyl-Coa])

d([Malonyl-Coa])/dt=α*(-β_2.kf*[Malonyl-Coa]) + β_3.kf*[Acetyl coa] + β_7.kf*[6PG]*[6 phosphogluconatedehydrogenase] + β_8.kf*[Acetyl Coa]*FASN)

d([fatty acid])/dt =α*(-β_5.kf*[fatty acid]-β_5.kr*TG-β_6.kf*[fatty acid]-β_6.kr*[Acetyl Coa])

d([Acetyl Coa])/dt =α*(β_6.kf*[fatty acid]-β_6.kr*[Acetyl Coa] - β_8.kf*[Acetyl Coa]*FASN)

d([6PG])/dt =α*(-β_7.kf*[6PG]*[6 phosphogluconatedehydrogenase] + β_9.kf*[6 phosphoglucono-siga-lactone]*lactonase)

d([Acetyl coa])/dt=α*(-β_3.kf*[Acetyl coa] + β_4.kf*Citrate)

d([6 phosphoglucono-siga-lactone])/dt =α*(-β_9.kf*[6 phosphoglucono-siga-lactone]*lactonase + β_{10}.kf*[glucose 6 phosphate]*[G6P dehydrogenase])

d([glucose 6 phosphate])/dt = α*(-β_{10}.kf*[glucose 6 phosphate]*[G6P dehydrogenase] + β_{11}.kf*Glucose - β_{13}.kf*[glucose 6 phosphate]-β_{13}.kr*[pyruvic acid])

d(Glucose)/dt = α*(-β_{11}.kf*Glucose + β_{12}.kf*Carbohydrate)

d(Carbohydrate)/dt = α*(-β_{12}.kf*Carbohydrate)

d([pyruvic acid])/dt = α*(β_{13}.kf*[glucose 6 phosphate]-β_{13}.kr*[pyruvic acid] - β_{14}.kf*[pyruvic acid])

d(pyruvate)/dt=α*(β_{14}.kf*[pyruvic acid] - β_{15}.kf*pyruvate)

d([citric avid cycle])/dt =α*(β_{15}.kf*pyruvate -β_{16}.kf*[citric acid cycle])

d(CO2)/dt =α*(β_{16}.kf*[citric acid cycle])

d(Citrate)/dt =α*(-β_4.kf*Citrate+ β_{16}.kf*[citric acid cycle])

d([6 phosphogluconatedehydrogenase])/dt = α*(-β_7.kf*[6PG]*[6 phosphogluconatedehydrogenase])

d(lactonase)/dt =α*(-β_9.kf*[6 phosphoglucono-siga-lactone]*lactonase)

d([G6P dehydrogenase])/dt =?α*(-β_{10}.kf*[glucose 6 phosphate]*[G6P dehydrogenase])

d(FASN)/dt =α*(-β_8.kf*[Acetyl Coa]*FASN)

Chapter 5

Conclusion

FAS articulation is related with an increased danger of breast cancer reoccurrence [35] and control of FAS gives chemoprotection; down direction of FAS causing breast tumor cell line to pretend more sensitive to chemotherapy drugs. Insulin-like growth factor (IGF)- I have been appeared to up direct FAS in malignant breast cancer cells and when FAS was suppressed, IGF-I intervened cell development was hindered [9][86][94]. The formation of palmitate from acetyl-CoA and malonyl-CoA is catalyzed by FASN. Acetyl-CoA-carboxylase is another critical enzyme in fatty acid formation whose function is to feed FASN with malonyl-CoA which is catalyzed to malonyl-CoA from the ATP-subordinate carboxylation of acetyl-CoA, and in this manner going about as the rate-limiting enzymes in the fatty acid formation pathway [103].

A comprehensive pathway was retrived and verified from literature and updated by introducing the missing enzymes and proteins from literature, verification was given in table 4.1 and 4.2. Then with the help of Protparam tool, all physio-chemical and ADME properties of all metabolites were estimated. LD50 value and toxicity were calculated by using Protox server tool and parameters for all metabolites were calculted by using these properties and parameter estimation equation of half life. As in normal metabolic pathways of cells, FASN express itself rarely because glycolysis was enough for compensating their energy demand. But in case of cancer, FASN is overexpression for compensating the energy demand

of abnormally growing cells. So model of overexpression of FASN was developed using toolbox in Simbiology MATLAB for the simulation of over expression of FASN.

There are numerous inhibitors of FASN in already working in Laboratory with best effects but some of them have high toxicity level. For designing a best drug with minimum toxicity the drugs which are FDA approved drugs are already available at drugbank,were used. After calculating all the physiochemical and ADME properties of drugs their five different cominations i.e. drug cocktails are made using Chemdraw tool. The properties of all drug cocktails were calculated using different tools i.e. swissadme, ACD/I-lab reports and protox server for calculating toxicity and LD50 values of cocktails. Depending upon the fitness value i.e. toxicity of cocktail, the best cocktail 2 choose as a drug and integrated into pathway showing up regulation of FAS gene. After the integration of dose with pathway, the expression of FAS shows down regulation.

However, in future it is possible to apply the same process on the Signaling pathway for controlling the regulation of FASN and other its key controllers e.g. HER-2, mTOR and SREBP-1 genes which are responsible directly for the production of FASN and level of its regulation. and along with toxicity, stability is also a important facto which can be focused in future work for the improvment of drug cocktails. As this is a in silico approach so in future work this drug cocktail can be tried in wet labs of clinics for therapeutic purposes. And PBPK modeling is also a innovative approach that can be applied for reducing the need of wet lab trial.

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