A Review on the role of Myc and miRNA in Glioblastoma Multiforme

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

The Department of Pharmacy

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Declaration

It is hereby declared that

1. The thesis submitted is my original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

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Abstract

Glioblastoma multiforme (GBM) is known as one of the most belligerent and malignant brain tumors in adults. Among the four grades of brain cancers, the glioblastoma is considered as the most infiltrative one. GBMs account for 60%–70% of malignant glioma. The median survival period of patients with such types of cancers usually exceeds from one year to fifteen months after treatment. Scientists are now approaching toward alternative ways of treating glioblastoma permanently. Different types of microRNA (miRNA) and the Myc oncogene play a vital role in regulation of multiple downstream targets to bring about alterations in gene mutation, chromosomal stability and inflection of tumor growth. This review paper delineates the role of Myc and microRNA in metabolism of glucose and tumor growth. Along with the possible utilization of C-Myc and miRNA, and how C-Myc and miRNA can perform the role of biomarkers and therapeutic targets for glioblastoma multiforme.

Keywords: Glioblastoma multiforme; C-Myc; miRNA; Therapeutic target; Malignant

Dedication

I dedicate this humble attempt to my ever so loving parents for their unending support and gracious perseverance towards me.

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To begin with, I would like to thank the Almighty for bestowing upon me with grace and mercy.

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Table of Contents

List of Figures

Chapter 1

INTRODUCTION

1.1 Glioblastoma

Glioblastoma or glioblastoma multiforme is known as the most fatal and aggressive form of primary brain tumors. This deadly disease accounts for about 52% of the entire primary brain tumors and about 20% of all intracranial tumors. According to the classification of brain tumors by The World Health Organization, there are about four classes of brain tumors, from grade I to grade IV depending on their morphological and predictable clinical behavior. These four classes are-Pilocytic astrocytoma (Grade –I), Diffuse astrocytoma (Grade-II), Anaplastic astrocytoma (Grade –III) and Glioblastoma (Grade-IV). Of which glioblastoma multiforme is the most lethal form of astrocytoma They might arise from ordinary brain cells or may develop from an existing lower grade astrocytoma. It is also known as astrocytoma that develops from a specific type of brain cell known as astrocyte. The Astrocytes are star –shaped glial cells that comprise the majority type of cells in the central nervous system. They are involved in carrying out metabolic exchange between neurons and blood and are also responsible for providing support to the neurons.

1.2 Appearance of the Tumor

Glioblastoma multiforme appears as an inhomogeneous mass with a hypodense center and a variable ring of enhancement encircled by edema on CT scan. Nevertheless the tumor may appear on different shape depending on the extent of hemorrhage, necrosis and its age. Glioblastoma comprises all the major features of cancer, which includes biological ability to sustain cell

proliferation, developing resistance to apoptosis by inducing angiogenesis, activating invasion and metastasis. This malignant cancer has also the capability of reprograming energy metabolism and thus causing to genomic instabitly and immune destruction. The medial survival rate of a patient suffering from glioblastoma is approximately one year to fourteen months from diagnosis. Glioblastoma multiforme has the short survival rate among all the gliomas due to its tumor resistance to adjuvant therapy.

Signs of this forceful malignant growth contain broad invasion and solid vascular multiplication into the encompassing brain parenchyma. The cerebral hemisphere of the brain is considered as the most frequent site of occurrence of glioblastoma. Almost 95 percentages of these tumors is tracked down in supratentorial region, while a minimal percent of tumors ensue in cerebellum, spinal cord and brainstem. This lethal tumor usually starts in the cerebral white matter, here it develops very swiftly and can become larger in size before depicting any cancer symptom. The tumor may spread into the meninges or ventricular walls, generating a high content of protein in cerebral spinal fluid accompanied by an increased number of lymphocytes. Glioblastoma consists of multiple cell types, which are capable of proliferating very swiftly and invade other parts of the brain.

Glioblastoma multiforme contains a minor group of auto-renewing and tumorigenic cancer stem cells known as glioma stem cells (GSCs). These cells are involved in tumor invasion, resistance to available treatments for glioblastoma and thus initiate tumor recurrence. This feature of glioblastoma accelerates its recurrence rate and hinders complete removal of cancer cells by treatment thus minimizing survival rate. As the growth of brain tumor accelerate, they often crowd out or affect normal brain structure and functions giving rise to symptoms such as headache, seizures, memory loss accompanied with behavior changes.

Brain cancer remains the reason of about 189,000 deaths worldwide, with glioblastoma being the most common type of ailment. An increased intracranial pressure and cerebral edema is considered as two major effects of glioblastoma that drives patients to death Age is perhaps one of the most significant prognostic factors in glioblastoma. The biology, pathogenesis and severity of glioblastoma are highly influenced by age.

1.3 Rationale of the Study

The center reason of the study is to analyze the various effects of oncogenes and transcription factors that aids on the development and suppression of tumor. Glucose plays a vital role in the development of cancer cells in our body. This study intends to identify how glucose and glutamine are incorporated in the development of glioblastoma multiforme in our brain.

Because the expression of microRNAs is involved with the genes related to gliomagenesis. By profiling of miRNA, the origin, prognosis, and response to therapy of glioblastoma can be identified. Mycs are potent oncogenes. They are concerned with controlling a diverse cellular function. This includes several developing characteristics of cell, for instance, cell growth followed by proliferation and differentiation along with programmed cell death. The expression of Myc is related with expression of grade -8 glioma. By controlling the expression of Myc oncogenes, severity level of glioblastoma multiforme can be altered.

1.4 Aim and Objectives of the study

The reason of this study is to gather a complete and absolute knowledge about the influence of various transcription factors such as Myc and miRNA,on the growth and suppression of brain tumor.

The objectives of the study include-

1) The pathogenesis of glioblastoma and involvement of various cellular components and transcription factors in the development of glioblastoma multiforme (GBM).
2) Identifying how molecular modification of oncogenes and transcription factors can such as myc and microRNA contribute in making glioblastoma treatment more efficient.
3) Compile information on identification of a new GBM (glioblastoma multiforme) specific contrast agent that might be beneficial in the treatment of glioblastoma multiforme.
4) Gather information on Identification of various treatment options that might cure GBM.

Chapter 2

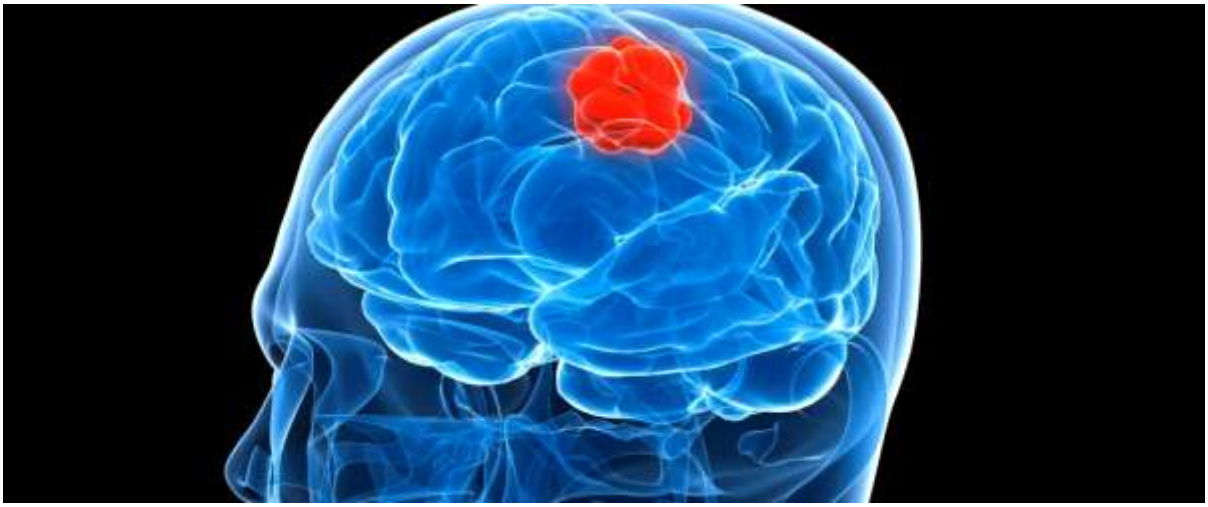
METHODOLOGY

This review paper has been prepared by extraction of multiple information from various scientific articles of various journals. The evidence, data and statistical values mentioned in this paper has been extracted from various scholarly articles. After conducting a comprehensive review of a number of articles, the vital information was traced, combined and synchronized accordingly. After going through continuous search, the most relevant articles were selected and examined for pinpoint information. Afterwards, based on the topic, the most accurate information was collected that suites accurately with the topic. Various online search engines and journal databases such as, Google Scholar, ACS Publications, Nature, SpringerLink, Wiley Online Library, PubMed, Science Direct were used for searching information as per requirement of the topic. The array of articles as per the need of this review paper**.**

Chapter-3

GLIOBLASTOMA AND ROLE OF MYC AND MICRORNA IN GLIOBLASTOMA

3.1 Description of Glioblastoma



Our brain is the most significant and sophisticated part of the body. It is responsible for controlling all the functions of our body. The human brain is the principal organ of the human nervous system and forms the central nervous system in by attaching with the spinal cord. The cerebrum, the brainstem together with the cerebellum completes the brain. Almost all the activities of the body is controlled by the brain including processing, integrating, and coordinating the information received from the sense organs, and making decisions as to the instructions sent to the rest of the body. The brain is contained in, and protected by, the skull bones of the head.

3.2 Brain Cells

It consists of two types of cell-the neurons and the glial cells. Glial cells make up 90 percent of the brain cells. Most of the supportive structures of the brain are provided by the glial cells. The glial cells include oligodendrocytes, astrocytes, ependymal cells and microglia.

The structural and supportive functions are controlled by the astrocytes. They are involved in synaptic transmission and are responsible for indication of brain injury. The ependymal cells are considered as the source of cerebrospinal fluid .These cells line the ventricles within the central nervous system.

The microglia perform the role of macrophages of the brain and gets activated in presence of pathogens within the brain. The salutatory conduction of the brain is modulated by oligodendrocytes. They conduct the process through axon myelination in the central nervous system. Three types of glial cell can give rise to tumors. Gliomas are categorized on the basis of glial cells involved in the tumor formation. (Kandel,Schwartz.et.al.2000.Mc Coy and Sontheimer 2010,Clarke and Barres 2013).

Glioblastoma is known as the most malignant form of brain cancer. It is considered as grade IV astrocytoma which is one of the rapidly growing types of central nervous system tumor. Glioblastoma might appear at any lobe of the brain including brain stem and cerebellum, but it is more prominently found in the temporal and frontal lobe. According to researchers, nearly 5 percent of all glioblastomas are initiated by hereditary reasons, while the remaining 95 percent glioblastoma cases occur due to some unspecified reasons. Glioblastoma is considered as one of the devastating brain cancers that characteristically results in death within the first 15 months after diagnosis.

In glioblastoma, a number of genetic mutations are involved in the formation of this cancer. The mutations criteria include-

- Inherited defects of DNA
- A cumulative consequence of exposure to carcinogens and other chemicals

- An exposure of ionizing radiation in high dose

The development pattern of glioblastoma is different from that of other cancers. Glioblastoma comprises of various cell types, creating obstruction in its treatment. Here cancer evolution is stimulated by somatic evoluation. This cancer occurs through an intricate network of molecular and genetic abnormalities giving rise to a significant alterations in chief signaling pathways. Unlike other cancers glioblastoma is difficult to treat. Location of the tumor is a vital fact in the treatment of glioblastoma. A complete removal of this tumor is almost impossible. The tumors formed in glioblastoma include thread like intense tentacles that remain extended into adjoining areas of the brain, leading to a high probability of reoccurrence of this disease. The glioblastoma tumor comprises of different types of cells. These different cells react differently to chemotherapeutic drugs while some also do not respond.

3.2 Available Treatment Options for Glioblastoma

There are several treatment options now available by the grace of advancement of medical science. After surgical resection, patients are prescribed a combined treatment of radiation and a chemotherapeutic agent, temozolamide. This treatment maximizes patient's survival for about three months. Temozolamide is a cytotoxic agent that gets active when it is within the alkaline tumor surrounding. The core function of temozolamide is to cause damage of DNA by methylating DNA bases at the N7, N3 and O-6 positions which afterwards hinders elongation of DNA strands causing mismatch of amino acid. This mismatching results in an unworkable cycling of DNA repair which eventually leads to termination of cell replication followed by death of the cancer cell. As infiltrative cancer cells show less response to temozolamide, so they are often administered along with radiation therapy for a complete treatment.

Radiation is a sort of anti-neoplastic therapy often used with chemotherapy especially in case of malignant cancers like glioblastoma. Radiation works by damaging DNA in active dividing cells resulting in a decrease of the mitotic activity of cells. Beside after surgery, radiation is also prescribed to glioblastoma patients when surgery is considered as unsafe or cannot be recommended. There are multiple methods of radiation. These includes-

1. Intensity modulated radiation therapy (IMRT)-This radiation based therapy is specifically used to treat tumor that is located in critical parts of the brain such as the area that is in control of the eye sight and the brain stem. Though it can also damage healthy tissue but it is quite effective against tumor as the radiation is directly focused at the tumors. The IMRT, functions by scattering the radiation beams into minor beams, the intensity of each of these beams can be altered according to what is required.
2. Stereostatic radiation therapy-In this type of radiation the radiation is focused on the outline of tumor through various angles. This radiation therapy is better in comparison to IMRT and surgery as this causes less damage to the neighboring healthy tissues and the therapy does not needs incision. The therapy requires shorter recovery period, less complications and less sensation of discomforts.

Among the above mentioned treatment options extensive surgical resection is preferred as the most effective process to prolong the persistence period of a glioblastoma patient. The feasibility of surgical resection highly depends on localization of tumor as well as infiltration, this is applied in cases when extremely specialized brain areas are involved. Areas involved in motor functions, senses and in control of speech are highly vulnerable. Moreover GBMs that are highly infiltrative are difficult to treat by surgery.

The current norm of care for high-grade gliomas patients incorporates not just helpful administration (for example hostile to tumor treatment), but on the other hand is comprehensive of giving compelling steady consideration to the patient. A compelling steady consideration involves dealing with the different signs and manifestations of the illness, which includes overseeing cerebral edema, seizures, gastrointestinal plot aggravations, osteoporosis, venous thromboembolism, intellectual disability and disposition issues (Norden and Wen, 2006). Suggestive alleviation of neurological manifestations is achieved by directing corticosteroids, anyway because of its significant results; it is normally tightened right off the bat in the treatment system. Dexamethasone, is normally the favored corticosteroid in these patients because of its low mineralocorticoid movement (Omuro and DeAngelis, 2013). In patients with seizures, Levetiracetam is frequently recommended as a result of its low poisonousness profile and no medication to tranquilize communications with chemotherapeutic specialists (Omuro and DeAngelis, 2013). Explicit restorative administration includes a medical procedure/careful resection of the tumor alongside radiation and attending adjuvant temozolomide (TMZ) treatment (Mrugala, 2013).

Regardless of several notable recent achievements in oncology, glioblastoma is still counted as one of the most complicated diseases to cure. There are myriad causes responsible for developing resistance to treatment in glioblastoma patients. Different types of cells like, stem- like tumor cells, brain tumor initiating cells (BTICs) and glioma stem cells assist in treatment failure in GBM by promoting tumor growth through sustained proliferation, angiogenesis, invasion of normal brain cells and self-renewal therapeutic resistance.

3.3-Genes, DNA and Cancer

Cells are the structural unit of all living organism. Almost every eukaryotic cell consists of a nucleus. This is known as the control center of the cell. The nucleus contains all the genome of the cell. The genome is the genetic material of the cell. The genome contains the DNA which is tightly packed into structures called chromosome**.** The chromosomes comprise of long chains of DNA and related proteins. Each DNA is approximately of 1.8 meters in length. Every human being carries 46 chromosomes, comprising of 22 pairs of autosomes and one pair of sex chromosomes: two X sex chromosomes for females (XX) and one X and one Y sex chromosome for males (XY).

The gene is considered as the basic physical and functional unit of heredity. The gene covers a specific sequence of nucleotides at a given position on a given chromosome that code for a specific protein (or, in some cases, an RNA molecule). The entities (genes) within the chromosomes are organized in such a way so that they obtain the capability of promoting cell functions. The integrity of genes and controls the activities of the cell are regulated by the nucleus.

Nucleotide Sequence in Genes:

Exons: These are the coding regions, which specify a sequence of amino acids. Introns: These are the non-coding regions which do not specify amino acids. Regulatory sequences: This determines when and where the protein is made (and how much is made).

3.4 How faulty genes lead to cancer

The genes are programmed to pick up mistakes that occur when cells divide. These mistakes are called mutations. Mutations can occur throughout our lives, during natural processes in our cells. Or they can take place because of other factors such as:

- Radiation and Tobacco
- Ultraviolet radiation from the sun
- Some substances in food
- Chemicals in our environment

Naturally the cells of our body are capable of repairing any faulty gene in our body. In this case p53 is responsible for carrying out such repair. When the damage is tremendous, the cell may self-destruct instead. The immune system may recognize them as abnormal and destroy them. This helps to protect us from cancer. But sometimes mutations in important genes cause a cell to no longer recognize instructions. This results in the destruction of apoptosis process thus leading the cells to multiply uncontrollably and leading to cancer.

Genes involved in cancer-

1-Genes that encourage the cell to multiply (oncogenes)

Oncogenes are known as commanding genes, their main function is to command the cells to multiply and divide. They have the potential to cause cancer. In tumor cells, they are often mutated or expressed at high level.

2-Tumor Suppressor Genes: Genes that stop the cell multiplying

Tumor suppressor genes are responsible for decreasing the rate of cell division. Initiate repair of DNA mistakes, or instruct the cells when to die (a process known as apoptosis or programmed cell death). When a tumor suppressor gene gets deactivated as a result of mutation, cells can grow out of control, which can lead to cancer. The tumor suppressor gene, p53 is also responsible for carrying out DNA repair.

Cancer is considered as a life-threatening ailment and the second major cause of mortality worldwide next to cardiovascular disease. According to a recent report by GLOBOCAN it is estimated that in 2018 there were about 9.6 million cancer related deaths globally and more 18.1 million cancer cases are newly detected. Among numerous categories of cancers, brain cancer is considered as one of the prominent causes of cancer related deaths. According to The American Cancer Society there are almost 120 types of brain cancers detected till now.

Brain tumors are abnormal growth of cells in brain. Like all cancers brain cancer is also a multistep process where cell proliferates in an uncontrolled manner independently of growth signals. Among different types of cancers brain cancer is considered as one of the most fatal type. Though incidence rate for brain cancer is not very high like lungs or breast cancer but the survival rate of patients having brain cancer especially grade IV glioblastoma is very less. The incidence rate of different types of tumor and their grades varies by different age group. In children and young adults, metastatic tumors are rare in children and primary brain tumors of low-grade that is grade 1 or 2 gliomas are more common. The adults above the age of 30 to 40 years are more prone of being affected mostly by metastatic brain tumors, in comparison to more than half of all brain

tumors. Among the four grades of brain cancer Glioblastoma is the most commonly occuring malignant primary brain tumor affecting elderly patients in most of the cases.

Glioblastoma can be classified into primary and secondary type. Primary glioblastoma refers to those types of tumor that first appeared as Grade IV glioblastoma. About 80% of malignant primary tumors originate from glial cells of the brain and are known as gliomas. Secondary glioblastoma refers to those tumors that develop from lower a lower grade astrocytoma. The secondary glioblastoma is more common in people between 45 to 62 years of age.

3.5 How Transcription factors are related to cancer-

Now a day a huge number of researches are going on based on the influence of transcription factors and various macromolecules. The crucial aim is to perceive the information about how transcription factors can be beneficial for therapeutic aspect for glioblastoma multiforme. Along with many other internal factors, the initiation and suppression of glioblastoma are closely associated with transcription factors like MYC and small non-coding RNAs like microRNA. Dysregulation or up regulation on their expression initiate various genetic changes from a series of pre-malignant or cancer initiation step to the progression step of cancer in glioblastoma multiform.

3.6 Introducing Micro-RNA

MicroRNAs have emerged as important modulator of tumor initiation and progression in several types of human cancer.MicroRNAs (miRNAs) are classified as important classes of gene regulators that performs many significant regulatory roles in variety of biological processes. MiRNAs are minute, highly conserved non-coding RNA molecules comprising 21-23 nucleotides in length. MiRNAs were first discovered by Victor Ambros and his co works in the year 1993.The

discovery of microRNA arose in an experiment where a small RNA encoded by the lin-4 locus was associated with a nematode *caenorhabditis elegans.* The experiment was conducted by modulation of the protein,lin-14.(lee et al.,1993).Similar to protein ,the gene encoding microRNA are contained in the nucleus of a cell. In the DNA each gene is transcribed by RNA polymerase 2 which give rise either a regulatory gene or a messenger RNA.

Since there discovery in 1993 thousands of microRNA were found to perform a vital role in several features of cellular functions such as cell cycle control, stemness along with differentiation. The primary function of microRNA is to regulate the expression of mRNA through translational expression and mRNA cleavage. Till now scientists has discovered at around 500 types of micro-RNA genes in the human genome and it is now believed that this small RNAs have a central function in diverse cellular and progressive process like cell cycle control, cell proliferation, apoptosis, stem cell division intracellular signaling, cell metabolism and many more. Over a decade, researchers have discovered multiple roles of miRNA in our body. They are capable of maintaining numerous metabolic processes by means of complicated mechanisms. MicroRNA mainly works by acting on key enzymes and transporters of metabolic processes. They are known as master regulators, regulating different transcription factors, oncogenes or tumor suppressor gene .One of the crucial functions of microRNA is to regulate messenger RNA (mRNA) for silencing of gene during cellular development. Micro-RNA is capable of turning off gene by inactivating mRNA.A single miRNA is capable of regulating a several target messenger RNAs (mRNAs).The regulatory processes of microRNA are accomplished through the RNA-induced silencing complex (RISC).

MiRNAs participation in regulating cancer cell metabolism through controlling the expression of genes. Almost 52% of Micro RNAs are more frequently found in cancer associated genomic

regions that are more vulnerable to damages or mutation (Calin*et al.*2004).An abnormal expression of miRNAs can lead to several complications in our body. Deregulation of micro RNA might give rise to tumerogenisis, metastasis, accompanied by other stages of cancer. Deregulation of micro-RNA is observed in various types of brain cancer like malignant glioma. These outcomes have powerful implications for the role of miRNAs in cancer. MiRNAs are located in body fluids, for example, plasma, urine, serum and in circulating tumor cells. They mainly exhibit their potential as potential biomarkers. Since they are readily available, non-invasive diagnostic and prognostic biomarkers. In myeloma genesis, definite miRNAs are abnormally expressed early and are capable of detecting serious diseases much more swiftly and accurately.

3.7 How MicroRNAs function as oncogene-

Oncogenes are specific type of cancer related genes that are capable of inducing cancer in humans. They actively promote tumor growth and invasion. Oncogenes are involved in various activities like embryogenesis, cell-proliferation and other important cellular processes.

There is a bunch of evidence that indicates that miRNAs also act as oncogenic miRNA. MiRNAs are also known as post-transcriptional level regulator, this particular and important function of miRNA allow them to contribute in cancer development and progression by modulating oncogene or tumor suppressor gene(TSG).Multiple miRNAs are involved in various cancers, including glioblastoma multiforme, lung cancer, pancreatic cancer and breast cancer. The regulatory functions of miRNA promote them to control over or under expression of proteins. In glioblastoma multiformemicroRNA-124, miR-137,miR-145,miR-128,miR-9,miR-17 and other micro RNAs perform a role in differentiation of glioblastoma stem cells by weakening their stemness

characteristics. There are many proven data that revealed the involvement of different types MiRNAs in several vital biological characteristics of glioblastoma, including cell proliferation, cell invasion , angiogenesis. They also contribute in various energy metabolism including glutamine and glucose metabolism.

3.8 Expression pattern of MicroRNA in Glioblastoma

The expression patterns of various miRNAs highly influence the prognosis of a disease. Significant researches have been carried out to understand the pattern of miRNA expression, their impact on glioblastomagenesis and patient prognosis.

1. Role of MiRNA -153 in Glioblsatoma in glutamine metabolism

Micro-RNA plays an important role in cancer etiology. A noticeable number of micro-RNA are related to the propagation of glioblastoma multiforme. In cancer cells, an aberrant metabolism of glutamine is often noticed. This is compulsory for the proliferation, growth, and survival of cancer cells. In glioblastoma multiforme, metabolism of glutamine in highly regulated by Micro-RNA -153. This in result resist the excessive production of glutamate. The enzyme glutaminase catalyses the formation of glutamate from glutamine, this reaction is known as glutaminolysis. MiRNA-153 regulates glutaminolysis through directly controlling the expression of glutaminase, though excessive expression of glutaninase may also withdraw the effect of mir-153 on glioblastoma cells.

In some cancers like hepatocellular carcinoma, non-small cell lung cancer, osteosarcoma MicroRNA-153 sometimes function as a tumor suppressor.MicroRNA-153 also restrict proliferation of cells and support apoptosis by limiting the expression of myeloid cell leukemia

sequence 1(Mcl-1),B-cell lymphoma 2(Bcl-2) and insulin receptor substrate sequence 2(Irs-2) in glioblastoma multiforme.

A group of reachers carried out some experiments based on the influence of expression level of miR-153 on glioblastoma. (Zhenyang *et al*., 2017).For conducting their experiment in order to inspect the potential role of micro RNA in the growth of glioblastoma several analytical methods were used.

In a laboratory experiment on micro RNA-153, researchers have discovered, a noticeable down regulation of micro-RNA-153 in glioblastoma tissues. Expression of mir-153 is significantly reduced in glioblastoma multiforme cell line. In glioblastoma mutiforme, an overexpression of mir-153 results in an increase of apoptosis thus minimizing cell growth and proliferation from a laboratory experiment it was showed that transfection of miR-153 expresses a reduced cell proliferation and increase apoptosis.

2. Micro-RNA-124 and miR-137

In glioblastoma multiforme and anaplastic astrocytoma the level of expression of microRNA -124 and microRNA-137 were discovered to decrease significantly in comparison to non-neoplastic brain tissues. These microRNAs also block expression of CDK6 mRNA, CDK6 protein and phosphorylated RB in glioblastoma cells (Silber et al.).The two microRNAs are specifically related to differentiation of glioblastoma multiforme stem cells, resist proliferation of GBM cell lines, and thus block tumor growth.

In an experiment conducted by a group of researchers on GBM specimens and its methylation status, reached to a conclusion that alteration in methylation in CpG island of miR-137 may contribute to its down regulation in glioblastoma multiforme. (Ariel et al).In those GBM specimens

they noticed that hypermethylation of CpG island influences the down regulation of miR-137 in those specimens in comparison to normal brain cells specimens. MicroRNA -137 possess the capability to hinder the self-renewal activity of glioma stem cells. Self-renewal or formation of secondary neurosphere is one of the main characteristics of cancer stem cells. These two features are connected with the tumor forming ability of glioma stem cells. The stem cells associated with cancer are also related with rapid progression of tumor and clinical outcome of patients.MicroRNA-137 is also involved in down regulating the expression of RTVP-1.RTVP-1 is a protein that stimulates migration of glioma cells, in the mesenchymal transformation stage of glioblastoma multiforme. The expression of this protein varies with the tumor grade. An over expression of RTVP-1 accelerates the self-renewal capacity of glioma stem cells, thus by silencing this gene miR-137 performs a vital function in blocking proliferation of glioma cells.

Similar to miR-137,microRNA-124 is also down-regulated in glioma.MiRNA-124 directly target CDK6 and inhibit their expression.CDK6 is responsible to regulate cell cycle progression and differentiation thus facilitates proliferation of glioblastoma multiforme cell.MiRNA -124 functions by inhibiting glioma cell invasion,neuresphere formation and tumorigenicity.They are also responsible to reduce stem cell marker expression in part by targeting a human embryonic protein SNA12 and the growth of CD133positive cells.CD133 is a known marker of cancer stem cell in glioblastoma.CD133 is capable of identifying cells that have the ability to promote neurosphere growth and form heterogeneous tumors.

MicroRNA 375-

MicroRNA 375 plays a vital role glioblastoma. An overexpression of miR-375 is associated with down regulation of RWDD3 in glioma cells and is also related to their mortality.

3.9 Introduction of Myc

The Myc is a type of transcription factor that controls several physiological processes such as cellular growth and division metabolism followed by apoptosis. Myc protein family is located in chromosome 8 and is believed to coordinate the expression of about 10 -15 percentage of all cellular genes. Myc belongs to a group of regulator gene or proto-oncogenes that code for transcription factor. Myc is very vital for the normal development of brain cells. By several experiments, it has been found that majority of glioblastoma have an increased level of Myc. (Annibali et al., 2014).

Initiation of cancer is also related with transcription factors. Activation or deactivation of transcription factors also contribute in the development of cancer, cell existence along with propagation and generation of tumor angiogenesis. The expression of Myc proteins in normal cells is strictly regulated by an important growth factor called mitogen. It is evident that due to loss of transcriptional control, mutation in Myc increases its expression. This might give rise to uncontrolled expression of genes concerned with proliferation of cells and thus leading to cancer. One of the reasons of such an expression might be the translocation of chromosome. And as a consequence of such translocation there would be a maximized amount of Myc protein expression which will eventually lead to more cell division and proliferation along will prohibition of the apoptosis process. This sort of amplification is also observed in large diffuse B-cell lymphomas and in a number of human epithelial tumors. Such type of Myc multiplication often leads to poor prognosis of the disease.

The family of Myc comprises of four related human gene- C-Myc, L-Myc,S-Myc and N-Myc.Among them, the C-Myc performs an exclusive role in the regulation and specifically in the

proliferation of glioma stem cells. In gliomas,C-MYC is co-related with the grade of malignancy. In grade I and II, the expression is lower while in GRADE III and in Grade IV the intensity of expression is higher.

Among the three types of Myc transcription factors, the C-myc is the most important one. It is known as the 'master regulator "which control several features of cellular growth regulation and cellular metabolism. The C-myc is a transcription factor for genes involved in growth control, differentiation and apoptosis of cell. The function of C-myc is cell specific,i.e. it depends on the physiological condition and type of a cell the how much of its apoptotic function will be regulated by C-myc.C-myc oncogene was first discovered as the cellular homolog of the retrovirus v-myc oncogene. Wilhelm Ellermann and Olaf Bang are considered as the pioneer in the discovery of C-myc oncogene. Later several scientists sought a better understanding of c-myc oncogene using their concepts

Over expression of C-myc drives voluminous sort of metabolic alterations in transformed cells and eventually leads to tumor. A number of dissimilar mechanisms (Spencer and Groudine, 1991), are responsible for deregulation of expression of C-Myc. This includes chromosomal translocation followed by amplification, activation of upstream growth stimulatory signaling cascades and maximized protein stability. Myc expression is deregulated in an extensive range of human cancers and is often related to aggressive, poorly differentiated tumors. An increased cellular proliferation is brought by a change in level of expression of gene families in glioma cells, is the consequence of an over expression of C-myc oncogene. After a study of nearly 30 years, C-myc is now considered to be involved as a key oncogene with a number of cancer types like glioblastoma, lung cancer, breast and colon cancer.

Right from the discovery of transforming oncogenes in the late 1970s, the biochemical study of cancer metabolism has been exceeded by efforts to detect the mutations that are involved in cancer initiation and progression. An increased glucose consumption, decreased oxidative phosphorylation and accompanying lactate production are considered as the key elements of metabolic transformation. These metabolic transformations are directed by activation of oncogenes. A number of malignant alterations are directed by an induced overexpression of the C-Myc.These changes influence the production of intermediated required for cell growth and division. A combined effect of oncogenes and tumor suppressor genes contribute in regulating various changes. Metabolic transformation results from a complex interaction between a generally hypoxic tumor microenvironment and multiple oncogenic mutations. These interactions results in an alteration in cellular metabolism that generally occurs in cancer or transformed cells.

3.10 Control of C-myc Expression

The expression and function of c-myc is specifically controlled by mitogenic signals in normal or untransformed cells. Characteristically C-myc messenger RNA is very short lived and is involved in driving of the proliferative function. Whenever there is an absence of positive regulatory signal a decrease in transcription takes place, this causes fall in the protein level and thus no proliferation of cells occurs. On the other hand, an increased function of C-myc is always noted in tumor cells. This amplified function might be the result of mutation in the gene itself or due to the induction of C-myc expression through an upstream of oncogenic pathways. C-myc inherits some unique features that aid in the correction of its oncogenic characteristics. One of the most unique features of C-myc is that, it has the ability of inducing apoptosis through several pathways, this feature of C-myc corrects it’s oncogenic properties. Another most important feature includes the stability C-

myc protein that helps in controlling C myc expression. In proliferating cells; the short half-life of C-myc plays a prominent role in gene regulation.

But, in some cases, posttranslational modification might also bring about an over expression of C-myc.For instance, mutation in the coding region of C-myc are usually seen inhuman lymphomas, specifically in the Thr58 phosphorylation site. The C-myc oncogene is overexpressed in a number of human cancers and is involved in the occurrence of at least 40% of tumors. The other members of the Myc gene group are also concerned with regulation of transcription in a wide variety of tumor types. The C-myc is capable of regulating multiple gene families that are involved in cell proliferation and cellular metabolism. In addition to that, C-myc is also concerned in stimulating several genes involved in biosynthesis of protein, cancer metabolism, transcription factors and cell cycle genes.

The genes that are involved in almost every crucial cellular functions are includes in the list of c-myc responsive genes. Along with the genes that are positively regulated by C-myc, the transcriptional activity of other genes, including cyclin D1 and carboxypeptidase D,is suppressed by C-Myc-Max complex or solely by C-Myc.C-myc plays a critical part in the regulation of multiple growth –promoting signal transduction pathways.

CHAPTER 4

IMPACT OF GLUTAMINE AND GLUCOSE METABOLISM IN GLIOBLASTOMA

4.1 Influence of Glucose and Glutamine Metabolism in Glioblastoma

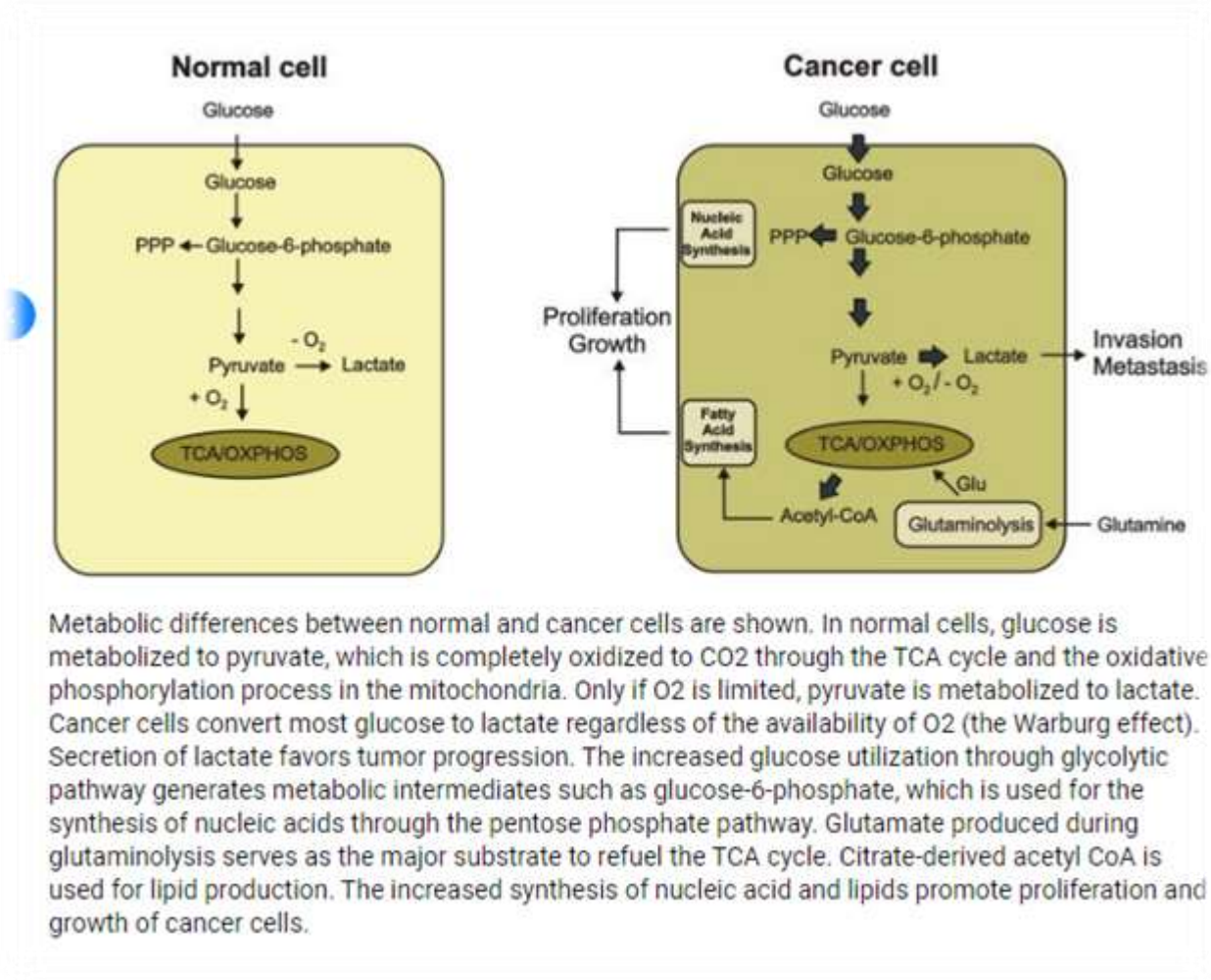
Glioblastoma also requires a constant source of energy and molecular resources like other cancers. Glucose and Glutamine are highly utilized by proliferating cancer cells to carry out their metabolic process in malignant cancers like glioblastoma multiforme. These are the two most essential growth component of both normal and cancer cells. Tumor cells change their pattern of metabolism in order to maintain unregulated proliferation and survival of cells. And the required nutrients to carry out these processes are supplied by glucose and glutamine .Cancer cells obtain energy from aerobic glycolysis, also known as catabolic process where one glucose molecule is converted into two lactate molecules, which produces a huge amount of lactate. The quantity of production of lactate depends on the malignancy of tumor. Aerobic glycolysis is regulated by oncogenic signaling pathways and tumor suppressors in both normal and cancerous brain cells. The activity of metabolic enzymes and glycolytic transporters changes when, any type of abnormal expression of these two genes are encountered in glioblastoma multiforme This lactate is then used for rapid proliferation of tumor cells. Glutamine is metabolized through glutaminolysis pathway. It is through glutamine metabolism, the cancer cells receive all the necessary amino acid, nucleic acid and glutathione for their proliferation. Thus targeting glucose and glutamine metabolism can bring about outstanding outcomes in cancer treatment.

Cancer cells initiate oncogenic mutation in order to increase nutrient uptake to support their proliferation. By catabolism of glutamine and glucose, cancer cells increase the production of

carbon molecules that act as building block for the cells. Moreover these carbon molecules also reduce the efficiency of normal cells to produce macromolecules and ATP generation. Otto Warburg ,almost 90 years ago, for the very first time discovered the fact that cancer cells consume glucose ten times more than normal cells for their growth and proliferation. Glucose is converted into lactate by the cancer cells even in presence of oxygen; this is known as Warburg Effect or aerobic glycolysis. Along with glucose, proliferating cancer cells also requires glutamine as a source of carbon. Eagle for the very first time discovered that cancer cells use 10 to 100 times more of glutamine than any other amino acid. (Tra-Ly Nguyen, Raúl V. Durán, 2018)

4.2 Difference between cancer cells and normal cells in the light of glucose and glutamine metabolism

Cancer cells differ from normal cells in a variety of ways.The most important difference is that the cancer cells are less specialized than normal cells. They continue to divide uncontrollably ignoring the apoptosis process which is necessary for the body to get rid of several unnecessary cells. Cancer cells are also capable of influencing surrounding normal cells, blood vessels and molecules in order to receive nutrients for tumor growth and development. For instance, cancer cells may encourage normal cells to form a network of blood vessels to supply oxygen and nutrient to tumors growth. Some cancer cells are also capable in hiding from the immune system. Thus the immune system can no longer detect their presence and they can continue to proliferate and invade and infect other normal cells. Cancer cells exhibit aerobic glycolysis in this process and glucose is converted into lactate followed by lactate fermentation. In this circumstance, formation of pyruvate dehydrogenase is decreased causing an increased glycolytic flux and minimizing the ability of pyruvate to participate in oxidative phosphorylation.



Metabolic differences between normal and cancer cells are shown. In normal cells, glucose is metabolized to pyruvate, which is completely oxidized to CO2 through the TCA cycle and the oxidative phosphorylation process in the mitochondria. Only if O2 is limited, pyruvate is metabolized to lactate. Cancer cells convert most glucose to lactate regardless of the availability of O2 (the Warburg effect). Secretion of lactate favors tumor progression. The increased glucose utilization through glycolytic pathway generates metabolic intermediates such as glucose-6-phosphate, which is used for the synthesis of nucleic acids through the pentose phosphate pathway. Glutamate produced during glutaminolysis serves as the major substrate to refuel the TCA cycle. Citrate-derived acetyl CoA is used for lipid production. The increased synthesis of nucleic acid and lipids promote proliferation and growth of cancer cells.

Figure 2 How the Process of Glycolysis differs in Normal Cell and Cancer Cell

4.3 The Unusual metabolism of cancer cells

Scientists are aware of the dissimilar metabolic actions of the cancer cells since 1950 when Otto Warburg for the very first time observed the high rate of glycolysis even in presence of sufficient oxygen to support OXPHOS. Cancer cells often depicts reprogramming of glutamine and fatty acid metabolism as a result of metabolic reprogramming. This metabolic reprogramming states some benefit to the cancer cells, these are, it ensures a sustainable supply of anabolic building blocks and supply the necessary amount of energy required for their association into macromolecules.

Generally, normal and cancerous cells follow similar metabolic pathways but in a different rate and the utilization purpose is also different. As for example, normal cells utilize the process of glycolysis chiefly to generate a very small quantity of ATP (2 molecules /molecule of glucose) and pyruvate. This produced pyruvate is then oxidized by the TCA cycle within the mitochondria and generate an equal amount of pyruvate to fill the required need in order to power the electron transport chain (ETC) and simultaneously to generate considerably more ATP (36 additional molecules).

On the other hand, cancer cells utilize glycolysis at an exaggerated speed for similar purpose of generating energy, along with this as a source of anabolic precursor for sustenance of rapid proliferation. The metabolic pathways are very much favorable and approachable that finally promotes the growth and survival of the cancerous cells. Such metabolic plasticity is very much essential to the tumor microenvironment as it supports the rapidity and the range to which the tumor microenvironment can alter. The result of such an unusual metabolic atmosphere is loss of normal apoptotic process that leads to an immense population of cancer cells. Some cancer associated metabolic reprogramming might produce metabolites that are capable in changing tumor behavior along with the expression profiles of gene. These effects can be active or passive, and the source of metabolites can either be the product of cellular respiration or "onco-metabolites"(Goetzman and Prochownik,2018).These so called onco-metabolites depicts noemorphic features and are assumed to get generated from mutation in mitochondrial enzymes.

For a proper maintenance of cellular homeostasis all living cells requires nutrients. These nutrients are obtained through various metabolic processes in the body. In an ordinary cell, glucose is converted into pyruvate by the process of glycolysis. This then enter into the tricarbooxalic cycle

(TCA) cycle to generate ATP through oxidative phosphorylation process.ATP is the main energy source required by all respiring cells .Our brain comprises of only 2% of our total body weight but it consumes of about 20% of oxygen of the entire body and 60% from the available glucose obtained from our everyday glucose intake. This is because our brain requires a constant stream of glucose for maintaining its proper function as it cannot store glycogen in it.In case of cancer cells, pyruvate is oxidized into lactate by glycolysis. This excessive amount of lactate brings about several changes in the cells. This vast amount of excretion of lactate lowers extracellular pH thus increasing the effect of some extracellular protease enzyme. This intensifies the invasiveness of the tumor and metastatic spread. Excess amount of lactate is also responsible for up regulating vascular endothelial growth factor and hypoxia inducible factor-1 alpha (HIF-1α).Hypoxia inducible factor 1 alpha is transcription factor sensitive to oxygen, this transcription factor positively controls glycolysis mainly in association with C-myc.Myc is deregulated in almost all types of human cancer. Presence of excessive amount of lactate also results in radio resistance in some tumors. In addition to that, tumor generated lactate might also influence surrounding fibroblasts to upsurge their synthesis and release of hyaluronan.Hyaluronan is a type of high molecular weight glycosaminoglycan which increases mortality and accelerates the spread of tumor cell.

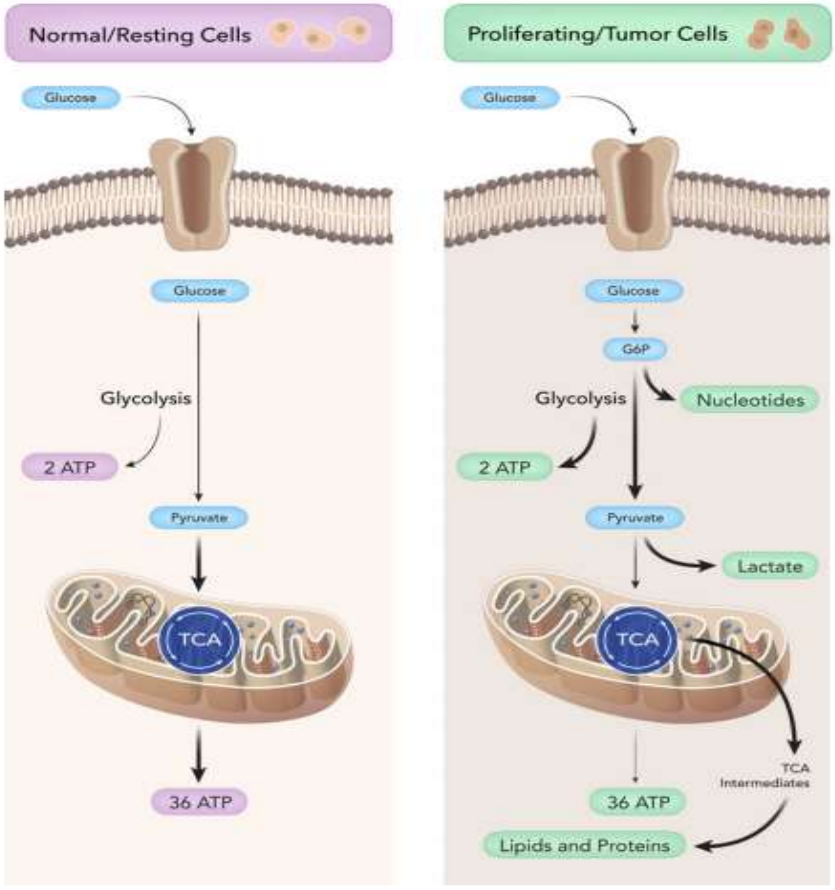


Figure 2 Showing the Difference between Resting Cells and Proliferating Cells

4.3 Utilization of glutamine in cancer cells

Glutamine metabolism is considered as a unique feature in conjunction with the Warburg effect in cancer. Cancer cells obtain energy and nutrients by glutamine metabolism for providing support required for cell growth and proliferation.

Among many amino acid, glutamine is the most abundant amino acid in plasma. Its concentration in blood is about 0.5 mmoles/litre.Glutamine is utilized by the cancer cells for their proliferation and surviving in stressful conditions. Glutamine acts as the most essential source of carbon, amino

acid and glutathione along with nitrogen that are basically utilized by proliferating cancer cells for nucleic acid synthesis.In glioblastoma cells glutamine is catabolized by various types of enzymes. These includes, glutaminase(GLS0, CAD or glutamine fructose-6-phosphate amidotransferase (GFAT).Most cancer cells exhibit a high dependency on glutamine and express an increased expression of SLC1A5.This is one of the major transporters of glutamine.

Glutamine acts as an anaplerotic source that means, it replenishes the amount of lost carbon that had been utilized from the TCA cycle to supply biosynthetic reactions. Through mitochondrial glutaminolysis, glutamine is transformed into alpha-ketoglutarate. At the initial stage of this reaction, glutamine is first deamidated to glutamate by the enzyme glutaminase. Then glutamate is again deamidated to alpha-ketoglutarate by the enzyme GLUD1or glutamate dehydrogenase (GDH)or aminotransferase. This then enters the TCA cycle to produce energy for the cells. The enzyme glutaminase that controls the entire glutaminolysis process consists of two isoforms,GLS1(kidney type glutaminase) and GLS2(liver type glutaminase).The isoform,GLS1 is mainly expressed in cancers and also up regulated in a wide variety of cancers. An increased level of GLS1 is also related to a higher disease stage and inferior prognosis. (Yu *et al.,2015).* In tumor cells, the activity of another enzyme like, glutamate dehydrogenase is also increased. Here, leucine acts as an allosteric activator of GDH. This increases the activity of GDH, thus increasing the production of alpha-ketoglutarate. Glutamine is associated in controlling of cellular signaling and act as a source of precursor for production of energy and macromolecule biosynthesis.

4.4 Role of microRNA in glycolytic metabolism in glioblastoma

MicroRNAs impart post transcriptional control on the expression of gene and along with it, plays the role of a critical regulator of aerobic glycolysis in glioblastoma multiforme. MicroRNAs are responsible for controlling the expression of various metabolic and regulatory genes. They are capable of regulating glucose uptake by controlling expression of glucose transporters (GLUT). Till now, scientists have discovered the involvement of specifically four microRNAs that are directly involved in modulation of expression of enzymes and glycolytic transporters in glioblastoma multiforme. Several cancers adopt the aerobic glycolysis process to fulfill their need of nutrient required for proper nourishment of cancer cells in response to micro environmental stress. In glioblastoma multiforme the rate of aerobic glycolysis is more in comparison to other types of cancers.

4.5-Warburge Effect

In tumors and other proliferating or developing cells, the rate of glucose uptake intensely increases resulting in the production of an increased amount of lactate, even in the presence of oxygen and fully functioning mitochondria. This process is known as the Warburg Effect. The mode of metabolism of cancer cells is considerably different from that of other cells during tumerogenesis and progression. An increased level of glucose metabolism is one of the key factors of cancer cells, even in aerobic conditions. The Warburg effect permit cancer cells to fulfill their enhanced demand of energy along with providing all the required biological materials utilized in the anabolic process such as synthesis of lipid and nucleotide .Along with that this effect is also responsible for

minimizing the production of reactive oxygen species in mitochondria, thus facilitating growth advantage for tumor.

Generally the effect comprises of four hypothesis.

- ATP/Glucose production
- Flux into other metabolic pathways
- Increase the pH, H^+ invasiveness
- Cell signaling

4.6 Role of microRNA in regulation of key enzymes of Warburg effect

MicroRNAs are considered to play a vital role in changing expression and activity of major glycolytic enzymes according to the need of tumor cells.

There are two ways in which these small non-coding RNAs regulate glycolytic metabolism in glioblastoma multiforme. Primarily they undeviatedly control the expression pattern of genes participating in glucose uptake and metabolism of glucose in glioblastoma multiforme. Among various micro RNAs, miR-106a controls glutamine synthetase 3(GLUT3), miR-143 regulates hexokinase II(HKII), let-7a and miR-326 regulate pyruvate kinase muscle isoenzyme(PKM II).Secondly, micro-RNA is also capable of controlling glycolysis indirectly by monitoring the signal transduction of receptor tyrosine kinase(RTK) through PI3K/AKT and RAS pathways that results in the upregulation of AKT activity as well as the expression of c-MYC.AKT and C-Myc

performs a role in accelerating the expression and function of glycolytic enzymes and transporters.MiR-106a is involved in the down regulation of expression of GLUT3 in glioblastoma multiforme. However the level of miR-106a is found lower in glioblastoma patients and is related with the malignancy of the disease. As the intensity of expression of miR-106a decreases the expression of GLUT3 increases. An increased expression of GLUT3 enzymes will enhance glycolytic metabolism. Thus, facilitating the cancer cells to receive more glucose for their survival. (Huda, Alan &Stuart,2017)

Along with miR-106a,miR-143is also down regulated in glioblastoma multiforme compared to other low graded brain cancer and normal brain. In glioblastoma, miR-143 is involved with the expression of another glycolytic enzyme, HKII. The expression of HKII increases with degradation of miR-143.An increased level of HKII is also involved with poor prognosis of the disease. miRNA-let-7a is associated in regulating another glycolytic enzyme,PKM2.This enzyme is known as the M2 isoform of pyruvate kinase(PK) and acts as the terminal glycolytic enzyme concerned with converting phosphoenolpyruvate to pyruvate. In glioblastoma multiforme, PKM2 is also targeted by another micro RNA,miR-326.A down regulation of this micro RNA is also observed in glioblastoma in comparison to normal brain. A decrease in the transcription of its host gene known as beta-arresting is thought to be responsible for a down regulation of miR-326 in glioblastoma. Thus, in glioblastoma, an over expression of this microRNA might lead to a reduced cellular proliferation, metabolic activity and a decreased level of ATP.MiR-326 facilitates its influence on tumor metabolism by inhibiting the expression of PKM2.

4.7 Role of microRNA in glutamine metabolism in glioblastoma multiforme

Cancer cells generate anomalous glutamine metabolism for promoting their growth, proliferation and survival. The entire glutaminolysis process that is the formation of glutamate from glutamine is catalyzed by the enzyme glutaminase (GLS). Micro RNA also plays a significant role in metabolism of glutamine. Dysfunction of microRNA can result in a significant impact in glioblastoma development and progression. According to the article 'MicroRNA-153 regulates glutamine metabolism in glioblastoma through targeting glutaminase 'it can be said that in glioblastoma tissues, there is a remarkable down regulation of micro RNA -153This micro RNA has been identified as a tumor suppressor in some cancers namely hepatocellular carcinoma, on-small cell lung cancer and many more. In glioblastoma multiforme, microRNA-153 control utilization of glutamine and generation of glutamate. MicroRNAs also play a function in the cellular function of GBM which includes cell death migration, invasion, proliferation, angiogenesis and drug resistance. They are also involved as a regulator in the glycolytic metabolism in glioblastoma multiforme.

4.8 How miRNA is involved in the regulation of glycolytic transporters and enzymes in GBM

As mentioned before, four types of micro RNAs are directly involved in modulating the expression of glycolytic transporters and enzymes, the expression of GLUT3 has been experimentally proved to be down regulated by miR-106a.The down regulation of miR-106a has been experimentally found more in glioblastoma multiforme in comparison to normal brain. This fact is also considered as one of the reasons of short time survival of GBM patients. The expression pattern of miR-106a varies with the grade of glioma. In high grade gliomas the expression level of miR-106a is lower while in low grade gliomas the level in very high. This fact also proves that down regulation of

miR-106a is also involved in promoting glycolysis and increases glucose flux. The increased rate of glycolytic flux is involved in the miRNA mediated suppression on GLUT3.

The three key enzymes of glycolysis are hexokinase, phosphofructokinase, and pyruvate kinase. Lactate dehydrogenase catalyzes the exchange of pyruvate to lactate. All these four compounds have been accounted for to be overexpressed in tumors, including cellular breakdown in the lungs, and can be directed by numerous oncoproteins to advance tumor multiplication, movement, and metastasis with reliance or freedom of glycolysis. Among the glycolytic enzymes, hexokinase II is mostly targeted by miR-143.The intensity of down regulation of Micro RNA 143 has also been reported more in glioblastoma multiforme in comparison to other grades of glioblastoma. PKM2 is another glycolytic enzyme, this enzyme is also regulated by the miRNA and let-7a. PKM2 is considered as the M2 isoform of pyruvate kinase (PK). PK is the terminal glycolytic enzyme, this enzyme is capable of converting phosphoenolpyruvate to pyruvate. The enzymatic activity of PKM2 is relatively less that leads to the gathering of upstream glycolytic intermediates that can be channeled into the biosynthetic pathways. The expression level of this glycolytic enzyme, is very low in glioblastoma and is totally absent in normal brain.

Micro-RNA-326, also illustrates some impact on PKM2.It is seen that, in glioblastoma multiforme, the overexpression ofmiR-326 or the knockdown of PKM2, reduces cellular proliferation, metabolic activity and levels of ATP.

4.9 Function of mi RNA in Regulation of Receptor Tyrosine Kinase (RTK)

Among different types of receptor tyrosine kinases, three RTKs, c-Met, PDGFRA and EGFR are targeted by microRNA. C-Met have been reported to be targeted by miR-410, which is down regulated in glioblastoma multiforme in comparison to normal brain and other low grades glioma. C-met is also a target of miR-144-3p, this micro RNA is also down regulated in glioblastoma. The expression of Mir-144-3p is inversely correlated with glioma WHO grade glioma and patient survival. Along with that, the expression of miR-34a in glioblastoma multiforme is suppressed by PDGFRA, This RTK is targeted by miR-34a in a negative feedback loop. Multiple links between miRNA and RTKs in terms of expression in glioblastoma were suggested by Kefas et al.(2008) and Webster et al.(2009).In their paper the proposed that EGFR is targeted by miR-7.,MiR-7 depicts a relatively decreased expression in glioblastoma.

4.10 miRNA in therapeutic treatment of Glioblastoma multiforme

There are two advantageous facts in targeting miRNAs as a therapeutic approach against GBM glycolytic metabolism. Firstly, by this way multiple genetic pathways can be targeted and secondly, the potentials to destroy glycolytic metabolism. These facts play a vital role in overcoming the hindrance in current therapy of glioblastoma multiforme. But due to certain drawbacks providing microRNA based therapies to glioblastoma patients is still under consideration. This is because of its low stability, potential of off-target effects, small -half life in plasma of miRNAs and absence of efficient delivery system.

Therapeutic targeting of microRNAs can be accomplished by the following processes by conducting different laboratory experiments in vivo and in vitro in order to demonstrate their therapeutic potentials.

There are different ways by which researchers tried to discover the process by which therapeutic targeting of microRNA can be accomplished. Among them, the replacement of down regulated microRNA is one of the processes. In this process, vectors representing similar microRNA are introduced in order to restore the expression of down regulated miRNA. For instance; a gradual decrease in the growth of tumor was observed when vector expressing miR-7 was administered systemically to orthotopic GMB xenografts. Re-expression of miRNA in glioblastoma cells also plays a role in enhancing the effectiveness of targeted therapeutics. For example, administration of miR-451 in combination with imatinib was reported to be significantly effective in suppressing the formation of glioblastoma neurosphere.

Along with replacement therapy, the therapy of inhibition of over expressed miRNAs is also considered as another method of therapeutic targeting of microRNA. In this process, microRNA antagonists also known as antagomiRs or anti-miRs are utilized to inhibit the function of miRNA. Antago-miRs are mainly antisense oligonucleotides which are complimentary and bind to, the mature miRNAs with the purpose of preventing its interaction with the miRISC complex. This fact was established by Corsten and his fellows in a laboratory experiment on mice. Corsten et al. (2007) transfected GBM cell lines with an anti-miR-21,and the matter was implanted into mice intracranial and it was then kept in observation for about 6 days. The result revealed a notable reduction in the tumor dimension. Along with that,anti-miR-21 also possess the capability of increasing the chemo-sensitivity of GBM cells as shown by Wong et al. (2012).Their team conducted a lab experiment of developing a TMZ (temozolamide)-resistant GBM sub-clones and

treated them with anti-miRNA and TMZ.The result showed that ,inhibition of miR-21 restrained the growth of the TMZ-resistant cells. The apoptotic rate of cells increases when cells are treated with a combination therapy of anti-miR-21 and TMZ in comparison with the treatment done solely with anti-miR-21.In another in-vitro study, it has again been showed that cells transfected with anti-miR-21 before to TMZ treatment shows an enhanced TMZ-induced cell death in comparison to cells treated only with TMZ.Moreover,inhibition of miR-21 increases the sensitivity of GBM cells to paclitaxel (taxol,an anti-microtubule agent),teniposide (VM-26,a topoisomerase II inhibitor) and 5-fluorouracil (a pyrimidine analogue).These are the three types of chemotherapeutic agents used for the treatment of GBM.

The above discussion showed multiple ways of involvement of microRNA in regulation of glycolytic metabolism in glioblastoma multiforme. Thus from these facts it can be concluded that microRNA based therapy can create a new approach and bring more advancement in targeting glycolysis and disrupt metabolic homeostasis in GBM cells.

Recently, scientists have discovered several significant role of microRNA in regulation of glycolytic metabolism in glioblastoma multiforme. MiRNAs regulate glycolytic metabolism by controlling the expression of glycolytic genes along with signaling proteins, in the PI3K/Akt pathway. These are responsible for regulation of glycolysis. There are multiple microRNAs that plays a role in the regulation of the PI3K/Akt pathway in GBM.Similar microRNAs are also functioned to control the components of glycolytic pathway directly in some other categories of cancer.

In GBM, miRNAs are either down regulated or up regulated. They play a vital role in suppressing or promoting the glycolytic metabolism in glioblastoma multiforme. From the above discussion,

it can be concluded that, inhibition of up regulated or the replacement of down regulated miRNAs could be a challenging therapeutic strategy to target glycolytic metabolism in glioblastoma multiforme.

4.11 Role of Myc in Glycolysis process

In glioblastoma multiforme, Myc smooth the progress of the renewal process of tumor by contributing in the tumor commencing cell reservoir involved directly with the maintenance of tumor. Among the most deregulated oncoproteins in all types of cancers Myc in very rarely mutated and is also responsible for regulating numerous metabolic functions. The wide-spread over expression of Myc in cancer recognizes that it is one of the most major transcriptional integrators of normal and oncogenic growth factor pathways.

Glycolysis is responsible for catabolizing glucose to pyruvate, which can be converted to lactate and secreted or enter the TCA cycle via either pyruvate dehydrogenase (to generate NADH and ATP) or pyruvate carboxylase. In GBM a significant increase in glycolysis is seen for energy production.

Fig. 1

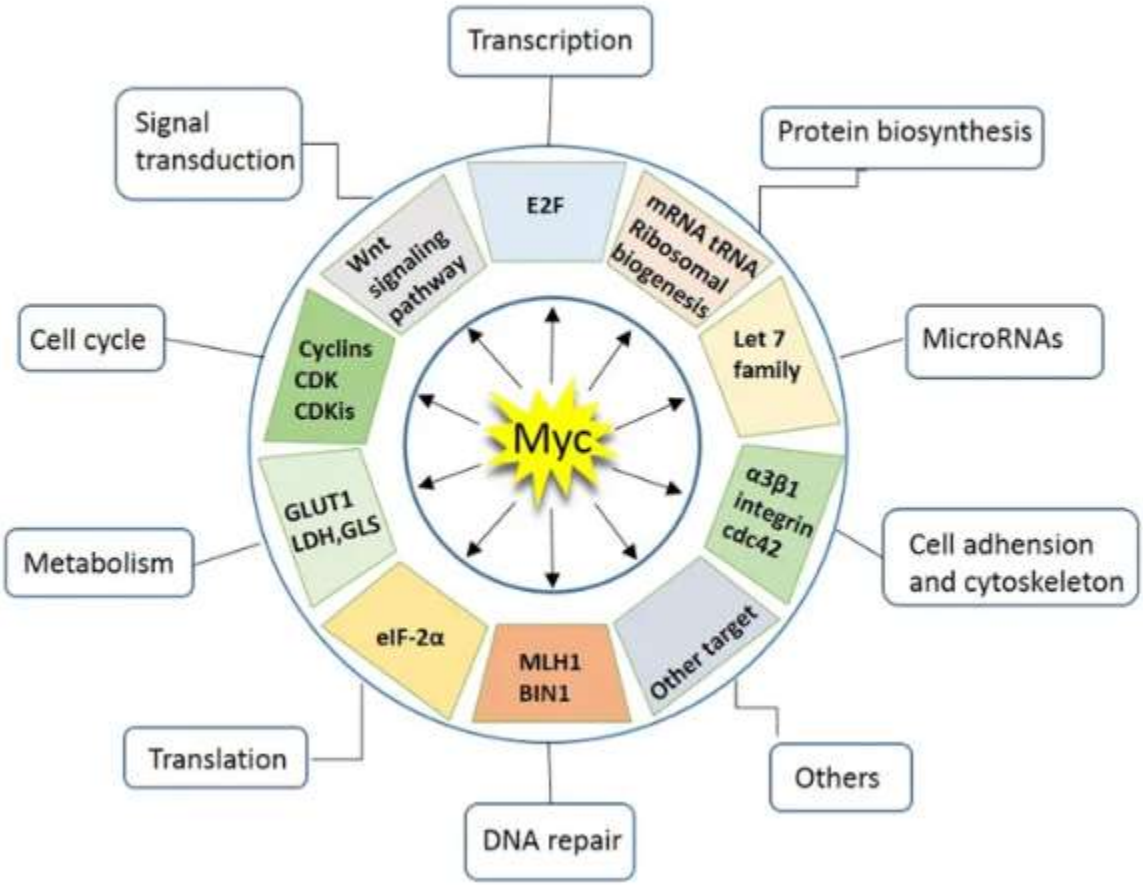


Figure 3 Functions of Myc

Myc controls a series of cellular functions. Myc controls a number of protein-coding or non-coding genes that are connected with separate cellular functions, including cell cycle, biogenesis of protein, metabolism, cell adhesion, transcription, signal transduction, and translation and many more. The series of Myc gene,(MYC,MYCN,MYCL)comprises of strong oncogenes that plays an important role in the pathophysiology of human cancer. The Myc oncoprotein is involved in the malignancy of cancer by multiple mechanisms. This includes promoting proliferation of cells, inhibiting differentiation, expediting chromosomal instability, accelerating cellular migration

along with provoking angiogenesis. In glioblastoma, Myc increases cell renewable capacity of glioma stem-like/tumor cells and assist in maintaining their tumorigenic potential.

High level amplification of the MYC and MYCN genes are observed in a subset of GBM.(Kensuke et.al;2016).That serves as a proof that Myc could be a promising therapeutic target in glioblastoma multiforme.

Deregulated Myc is also concerned with increasing the rate of glycolysis and glutaminolysis in cancerous cells. These enhanced rate promotes increased biosynthetic demand of rapidly proliferating cancer cells .Kensuke and his team in the in the article '*Myc-driven glycolysis is a Therapeutic target in Glioblastoma'* confirmed that, Myc is responsible for increasing glycolytic instability in GBM cells and also discovered that Myc generates a dependency on glycolysis for survival. To conduct their experiment, they used a panel of patient-derived GBM tumorsphere lines and was successful in deriving the concept of NAD and NAMPT(nicotinamide phosphoribosyl-transferase) involvement in glycolysis inhibition. From their experiment, they derived a result that small molecule inhibitors of the following enzymes own the potential for quick translation in a genetically defined subset of GBM patient.

C-Myc is known as the principal regulator or master regulator of glutamine utilization to ensure the cellular access of glutamine for replication of DNA in normal cells. It initiates the transcription of glutamine transporters such asSLC1A5/ASCT2, and endorses the expression of glutamine catabolized enzymes, that includes, glutaminase 1 (GLS1) and carbamoy*l*-phosphate synthetase 2 - aspartate transcarbamylase - dihydroorotase (CAD), in order to promote the uptake of glutamine by transforming glutamine to glutamate. But in case of cancer cells, this transcription factor is often up regulated thus hampering the consumption of glutamine which results in deregulation of cell cycle resulting an increased proliferation of cancer cells.

A few miRNAs are differentially communicated in an assortment of malignancies contrasted with comparing solid tissue. A portion of this miRNAs have been appeared to tweak oncogenes and tumor silencers, just like the incident for GBM. Thusly, miRNAs could hold an extraordinary potential later on treatment of this ailment. Due to its oncogenic properties in neoplastic cells, c-Myc has become an intriguing and doable objective for novel treatments of an assortment of human malignancies identified with c-Myc. It has been demonstrated that few little particles focus on the record of c-Myc quality straightforwardly or the c-Myc downstream pathway. Particularly, G-rich locale of c-Myc advertiser has become a promising objective. Numerous reports show that low-sub-atomic weight mixes can possibly be formed into remedial medications in individualized malignancy treatment. Indeed, even with the advances in the field of medication plan and in the systems hidden the c-Myc overexpression in tumor cells, it is as yet hard to acquire exceptionally explicit and dynamic enemy of malignancy drugs. Through the ID of different little atomic mixes that meddle with c-Myc, it very well might be conceivable to create novel and viable remedial specialists to treat disease.

4.12-Influence of Overactive MYC in Glioblastoma cells

Erroneous activation of MYC may lead to aerobic glycolysis in cancerous cells (“Warburg Effect”) in segment by inducing expression of major regulators of glycolytic pathway. In order to evaluate the effect of overactive Myc on glycolysis in GBM cells, an experiment was conducted using MyC-transduced U87 glioblastoma cells. (Tateishi et al.; 2017).In their experiment, lentivirus were used carrying either MYC or GFP driven by an active cytomegalovirus promoter to infect U87 glioblastoma cells for generating U87-Myc and U87-GFP cell lines. For determining the flux using

glycolysis, both U87-Myc and U87-GFP were cultured in normoxia with [U-C-13] glucose for 6 hours and the enrichment of isotope in glycolytic metabolites was resoluted. The entire experiment was directed through capillary electrophoresis time of flight mass spectrometry (CE-TOFMS). (Tateishi et al.;2017).It was found that the quantity of C-13 labeled glycolytic intermediates glucose 6-phosphate (G6P),pyruvate in addition to lactic acid, fructose 6-phosphate and DHAP were significantly amplified in U87-Myc in comparison to U87-GFP.This clearly indicated that Myc overexpression is performs a function in glycolytic flux. Along with that, overexpression of Myc is also concerned with a significant increase in the appearance of glycolytic enzymes HKII, LDHA and PKM2 in U87 cells. (Tateishi et al.; 2017).

Another experiment was carried out using glucose free solution to discover the effect of glucose in U87-GFP cells and U87-Myc cells. After assessing some experiments, Tateishi and his co-researchers reached into the conclusion that U87-GFP cells sustained for a significantly longer period in glucose free culture solution in comparison to U87-Myc cells. This clearly indicates that in an overexpressed state Myc gets dependent on glucose for growth. In addition to that, on inhibition of the glycolysis process using 2- deoxyglucose (2-DG) (which is a small molecular inhibitor of hexokinase (28),)in U87-Myc and U87-GFP,it was discovered that with glucose deprivation,U87-Myc were found more complex to glycolytic inhibition with 2-DG in contrast to U87-GFP.

In the above two experiments it was found that an overactive MYC may lead to an abnormality in metabolic programing in glioblastoma cells. The aberration in metabolic programing is responsible for increasing the glycolytic flux in cells and might cause alteration of NAD+ metabolism. It was also found that Myc-driven GBM cells are complex to inhibition of the Myc-induced enhanced glycolytic drive, and identified NAMPT inhibitors, that weaken glycolysis by reducing NAD+

required for the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) step, as a selective and extremely effective strategy for MYC/MYCN amplified glioblastoma multiforme. At the end, these founding shows that an overactive Myc creates an altered metabolic state in melanoma cells that can be destroyed by inspecting their dependence to definite metabolites.

4.13 Role of C-myc in glioblastoma

C-myc belongs to the class of proto-oncogene. The expression of C-myc is closely associated with the malignancy. It has been found that, in Grade I and II glioma, the expression of C-myc is very low while in Grade III and IV tumors, the expression of C-myc in considerably higher.

An experiment was conducted based on the expression of various myc,(C-myc, N-myc, and L-myc proto-oncogenes) using samples collected from adult glioblastoma patients.The aim of that experiment was to discover the prognostic significance of myc in glioblastoma..(Herms.et.al; 1999).

Findings showed that out of 78 percentage of tumors communicated C-myc m-RNA, 84% max m-RNA, 57% N-myc m-RNA, and 57% L-myc m-RNA. In that test, the postsurgical endurance of patients after operation than 60 years old and that of patients under 60 years old were examined separately. There was no huge contrast in postsurgical endurance in both age bunch between the patients whose tumors connected either C-myc, N-myc, or L-myc, individually, and those whose tumors didn't display that trademark. A distinction in postsurgical endurance, in any case, was found in the more than 60-year age bunch between patients who developed tumors connected to an equivalent or lesser degree than C-myc and those whose tumors communicated max to a more prominent degree than C-myc or neither max nor C-myc. And at long last it was discovered that,

the biologic conduct of glioblastomas in more seasoned patients may rely upon the family member, yet not on irrefutably the substance of the C-myc protein and interfacing proteins.

4.14. How Glutamine metabolism is controlled by C-Myc

Glutamine is predominantly used by the malignant growth cells as a wellspring of nitrogen and other nutritive substances to conduct the process of biosynthesis of cancer cells. Notwithstanding that it is utilized as a substrate for anabolic cycles in malignant growth cells. Along with glucose, glutamine also provides additional energy for the development and expansion to cells. Glutamine metabolism is considered as a significant mitochondrial feature in malignant cells, a huge amount of glutamine is utilized for the production of ATP and lactate. An-initiated overexpression of C-myc encourages the declaration of qualities fundamental for cells that are engaged with glutamine catabolism. Catabolism of glutamine advances an expanded cell necessity for protein and nucleotide biosynthesis. C-myc subordinate glutaminolysis brings about reprograming of mitochondrial digestion. This drives the mitochondria to rely upon glutamine to support the cell feasibility and anapleurosis of the carboxyl corrosive (TCA) cycle. In some human tumors the utilization of glutamine is seen in such a degree that it surpasses the flowing plasma glutamine levels. Some current investigations portrays that malignancy related changes in the digestion of glutamine and glucose are strongly affected by C-myc articulation. (Mill operator et al., 2012).More explicitly, the showing of persevering C-Myc-subordinate hypoxic digestion of glutamine, even without glucose, demonstrates that this is a significant impact of C-Myc articulation. Without a doubt, in changed cells, over articulation of C-myc is liable for the contemporaneous conversion of glucose into lactate and glutamine oxidation in TCA cycle. In a condition with a diminished inventory of oxygen with an extreme focus of C-Myc,a significant

measure of glucose gets changed over to discharged lactate, in this condition glutamine is constantly used in the TCA cycle. This glutamine is then utilized by the glioblastoma malignancy cells for its endurance. Under hypoxic condition, the pace of transformation of glutamine into glutathione is expanded. Glutathione in a significant decreasing specialist for managing the amassing of mitochondrial receptive oxygen species (ROS).This articulation underpins the way that restraint of glutaminase is successful in slaughtering hypoxic glioblastoma malignancy cells in vitro and postpones tumor xenograft growth. The C Myc is additionally worried in modifying glutamine digestion by transcriptionally subduing the miRNAs, miR-23a, and miR-23b.This brings about an improved articulation of the mitochondrial protein, glutaminase (GLS).The result of an expanded mitochondrial glutaminase is an upregulation of glutamine catabolism. This lead to an expanded measure of glutamate which is additionally used through the TCA cycle. An expanded glutamine catabolism may likewise fill in as a substrate for glutathione blend. Glutamine is a flexible supplement needed for endurance and development of a conceivably huge subset of tumors. One significant thought is that not all malignancy cells require an exogenous stock of glutamine. Work throughout the following quite a while should deliver a more exact image of the sub-atomic determinants of glutamine enslavement and the ID of death pathways that execute cells the catabolism of glutamine is debilitated. Progression of glutamine-based imaging into scientific practice ought to before long make it conceivable to separate tumors that take up glutamine from those that don't. At last, the improvement of protected, high-intensity inhibitors of key metabolic hubs ought to encourage restorative regimens highlighting restraint of glutamine digestion. Tumors likewise display variable degrees of glutamine digestion in vivo. An investigation of orthotopic gliomas uncovered that hereditarily assorted, human-inferred tumors took up glutamine

in the mouse mind yet didn't catabolize it. Or maybe, the tumors integrated glutamine all over again and utilized pyruvate carboxylation for anaplerosis.

Contemporary improvements in glutamine-based imaging, combined with the fruitful utilization of metabolic inhibitors of glutamine in mouse models of disease, raised the thought of designing a treatment option concerned with the use of glutamine. A major test will anticipate which tumors are well on the way to react to inhibitors of glutamine digestion. Beginning proof recommends that metabolic reinventing additionally encourages protection from the norm of-care treatments used to treat GBM.

4.15-C-Myc is a therapeutic Target

C-Myc depicts multiple role in glioblastoma multiforme. This can be a beneficial healing target for managing glioblastoma multiforme. Deregulation of Myc proteins are seen in brain tumors. This is more frequently found in embryonic tumors that affect children. Numerous perceptions have uncovered how the variations of these pleiotropic Myc record factors give inception, support, or movement of tumors. Numerous tumors display a dependence on Myc oncoproteins. This is additionally evident in Myc-driven medulloblastoma models in which hindrance of Myc will cause a relapse and cell senescence of the whole tumor. In gliomas, suppressor qualities like p53 and Pten gets inactivated, whereas, the C-myc is basic for tumor upkeep, and C-myc restraint will smother the tumors by advancing separation of the glioma cells. Clearly Myc proteins are approved focuses for disease therapies, thus by focusing on these record factors that need clear restricting spaces have consistently indicated troublesome, and utilizing short meddling RNAs (like explicit short clasp RNAs) that target Myc straightforwardly isn't yet a treatment choice for patients. There

are, in any case, little atomic medications that restrain Myc-Max collaborations that are powerful in malignancies similar to human intense myeloid leukemia.

Chapter 5

CONCLUSION AND FUTURE PROSPECTS

This review paper emphasizes on the influence of transcription factors like miRNA and Myc in metabolism of glucose and glutamine in glioblastoma multiforme. The paper also comprises the involvement of these two transcription factors in the progression and treatment of the brain cancer, glioblastoma multiforme. Unfortunately, the existing treatment for this disease available to patients are not that much effective. Due to the complex biology of this cancer a complete curable treatment for this disease has not been yet discovered. However, surgical approaches, adjuvant chemotherapy and radiotherapy shows some development in survival and standard of life of the GBM patients but the prognosis is still depressing. The diffusely infiltrative nature of GBM has reduced the effectiveness of the prevailing clinical approaches. Thus, by targeting a gene and protein that mediate infiltration new approaches in the treatment of this disease can be discovered. This will facilitate the clinicians to find a better tool for intraoperative visualization of GBM. We hope that transcription factors and miRNA and Myc can play a vital role in improving the existing treatment options for Glioblastoma Multiforme in future.

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