European Review for Medical and Pharmacological Sciences 2015; 19: 1630-1639

Role of micro-RNA (miRNA) in pathogenesis of glioblastoma

J.W. LUO, X. WANG, Y. YANG, Q. MAO

Department of Neurosurgery, West China Hospital, Sichuan University, Wuhou District, Chengdu, China

Abstract. – Glioblastoma (GBM) is a very lethal form of human brain cancer, which is characterized by rapid diffuse, infiltrative growth and high level of cellular heterogeneity. Such cancer patients usually survive for one year under treatment. Recently, the role of small non-coding RNA known as microRNAs (miRNA), have been suggested to be involved in the pathogenesis of glioblastoma, as miRNAs play a critical role in the tumor-forming processes. The change in expression levels of several miRNAs has been found in GBM patients within last 10 years. It is evident now that impairment of miRNA regulation is one of the key mechanisms in GBM pathogenesis. The miRNA deregulation is involved in many processes, such as cell proliferation, cell cycle regulation, apoptosis, invasion, glioma stem cells behavior, and angiogenesis. GBM is also known as Grade IV astrocytoma, a rare disease with an incidence of 2-3 cases per 100,000 people in Europe and North America, and 50% with GBM die within 1 year, while 90% within 3 years. The treatments of GBM involve chemotherapy, radiation and surgery. The median survival with standard radiation and chemotherapy with Temozolomide is 1 year and 3 months, and median survival without treatment is four and a half months. In this article, symptoms of GBM, treatments, the role of miRNAs, gene expressions, types of miRNAs, neoplasms and glioblastomas, the miRNA biogenesis pathways, deregulation of miRNAs, and care of GBM have been described.

Key Words:
Glioblastoma, miRNA, Pathogenesis, Temozolomide, Genetics, Differentially expressed genes.

Introduction

Glioblastoma (GBM) is the most lethal form of human brain cancer, and is characterized by rapid diffuse and infiltrative growth and high level of cellular heterogeneity related with therapeutic resistance[1]. The brain cancer patients generally survive for one year under treatment. A considerable progress has been made in understanding of the role of small non-coding RNA molecules, known as microRNAs in the pathogenesis of glioblastoma. miRNAs play a critical role in the multiple steps of tumor-forming processes. The modulation of miRNA expression, using either antisense oligonucleotides or precursors mimic sequences, show a marked potential for application in GBM-targeted therapies either per se or in combination with chemo- or radiotherapy. Diagnosis, biomarkers have been developed for prognosis and prediction, and treatments with advances in microRNA therapeutic research *in vitro* and preclinical studies of GBM patients. The microRNAs are endogenously expressed regulatory non-coding RNAs[2]. The changes in expression levels of several miRNAs have been observed in GBM patients. Since last 10 years, the function and direct mRNA targets for these miRNAs have been studied and it is evident now that the impairment of miRNA regulatory network is one of the key mechanisms in GBM pathogenesis. The miRNA deregulation is involved in many processes, such as cell proliferation, cell cycle regulation, apoptosis, invasion, glioma stem cells behavior, and angiogenesis. It is suggested that the miRNA functions in GBM are involved with an emphasis on its significance in GBM oncogenic cell signaling and its potential to serve as a disease biomarker and as a new therapeutic target for cancer. The GBM is also known as Grade IV astrocytoma[3]. The GBM is a rare disease, with an incidence of 2-3 cases per 100,000 in Europe and North America. About 50% of the patients diagnosed with GBM usually die within a year, while 90% within 3 years. The treatments of GBM involve chemotherapy, radiation and surgery. The median survival with standard radiation and chemotherapy with Temozolomide is 1 year and 3 months, and median survival

Corresponding Author: Qing Mao, Ph.D; e-mail: qingmao428@163.com

without treatment is 4½ months. However, no randomized controlled trial studies have been done, but surgery remains the standard treatment of cancer.

Symptoms of GBM

The common symptoms of GBM include, seizure, nausea, vomiting, headache, memory loss, and hemiparesis. The single most prevalent symptom is a progressive memory, personality, and neurological deficit due to temporal and frontal lobe involvement. The heavy smoking and chronic alcohol consumption is also a possible risk factor. The GBM has been related with the viruses SV40, HHV-6, and cytomegalovirus. There is a small link between ionizing radiation and GBM. It is also believed that there may be a link between polyvinyl chloride (commonly used in construction) and GBM. In 2006, research showed a link between brain cancer and exposure to lead in the work-place. The GBM is usually formed in the cerebral white matter, which grows quickly and can become larger in size before producing cancer symptoms. Less than 10% of GBM form more slowly following degeneration of low-grade astrocytoma or anaplastic astrocytoma, which are called secondary GBMs and are more common in people (aged 45 to 62 years). The GBM tumor may extend into the meninges or ventricular wall, leading to high protein content in cerebrospinal fluid (> 100 mg/dL), and an occasional pleocytosis of 10 to 100 cells, mostly lymphocytes. The cancer cells carried in the CSF can spread slowly to the spinal cord or cause meningeal gliomatosis. The metastasis of GBM beyond the central nervous system (CNS) is unusual. About 50% of GBMs occupy more than one lobe of brain hemisphere or are bilateral. The tumors of this type usually arise from the cerebrum and may rarely exhibit the infiltration across the corpus callosum, producing a butterfly (bilateral) glioma. The tumor may take on different appearances, depending on the amount of hemorrhage, necrosis, or its age. The CT scan usually shows an inhomogeneous mass with a hypodense center and a variable ring of enhancement surrounded by edema. The mass effect from tumor and edema can compress the ventricles and cause hydrocephalus. The cancer cells with stem cell-like properties have been found in GBMs. This can be a cause of their resistance to conventional treatments, and high recurrence rate. A biomarker for cells in GBMs that exhibit cancer stem cell properties, the transcription factor Hes3, has been shown to regulate their number when placed in nerve cells culture.

When GBMs are viewed in MRI scan, these often appear as ring-enhancing lesions. The appearance is not specific, because other lesions such as abscess, metastasis, tumefactive multiple sclerosis, and other entities also show a similar appearance. A positive diagnosis of a suspected GBM on CT or MRI scans requires a stereotactic biopsy or craniotomy with tumor resection and pathologic confirmation. The treatment and prognosis GBMs include symptomatic therapy, palliative therapy, surgery, radiotherapy, chemotherapy, gene transfer, and microRNA screening of plasma. The protein therapeutics - APG101 treatment without drug is useful for 2 years for GBM. The immunotherapy can be applied for the GBM treatment. For prognosis, the median survival time from the time of diagnosis without any treatment is 3 months, but with treatment survival of 1-2 years is possible. The increasing age (> 60 years) carries a poorer prognostic risk. The brain cancer patient's death usually occurs due to a cerebral edema or increased intracranial pressure. The patients with glioblastoma multiforme (GBM) have a very poor prognosis, median survival time from diagnosis is 3 months, and an increase of molecular and cellular mechanisms involved in the pathogenesis of GBM[4]. Thus, a new research and development strategy is required to improve the outcome of GBM patients. The GBM is the most common primary brain tumors in adult population and the causes and pathogenesis of this disease have not yet been clearly identified.

Expression of microRNAs in GBM

A total of 1,236 significant and differentially expressed genes, and 30 pathways enriched in the set of differentially expressed genes among 243 tumor and 11 normal samples have been identified[5]. Ninety-seven differentially expressed microRNAs among 240 tumor and 10 normal samples were also identified. Twenty two of which were reported to affect glioblastoma and 50 were implicated in other cancers and brain diseases. The gene expression was regressed on microRNA expression in 237 tumor tissues and 10 normal tissues. Two experimentally validated microRNA targets and 1,094 miRNA-target gene pairs were found in the data sets which were predicted by microRanda Algorithm. Eight of the target genes were tumor suppressor genes and 3 were oncogenes. The function analysis of target

genes further suggested that miRNAs were most frequently targeted genes related with cell signaling and nervous system of the brain cancer patients. These findings are important clues to further study the carcinogenic processes in the glioblastoma patients. The GBM is the most aggressive type of primary brain tumor, and accounts for 52% of all primary brain tumor cases and 20% of all intracranial tumors. In the past 20 years, the molecular mechanism, genetics and treatment for the pathogenesis of GBM have not been known clearly. Recent improvements of high-genomic technology have shown that it is now feasible to survey human cancer genome. The Cancer Genome Atlas (TCGA) catalogue which discovered major cancer-causing genomic changes in large cohorts of human tumors through integrated multi-dimensional analyses have been reported.

The microRNAs are single-stranded short coding RNA molecules of about 22 nucleotides in length, which repress gene expression by binding at the 3'UTR region of target gene. The expressions of miRNAs are highly different in organ development and tissue differentiation. Many miRNAs have been found to associate with apoptosis and cancer. This suggests their function as oncogene or tumor suppressor gene. In this study, the expression levels of 470 human miRNAs were performed in GBM and indentified a group of miRNAs whose expression is significantly changed in the brain tumor. The significant changes in gene expression and pathways related to GBM have also been identified. All types of research data were obtained from TCGA project[5] and the Website address is: (http://cancergenome.nih.gov/dataportal/data/about/ website). The gene expression microarrays were performed on Affymetrix HT Human Genome U133 Array Plate Set by MIT, Boston, MA, USA. The level three data gave calls for genes per sample after Probeset-level and Gene-level Robust Multiarray Analysis (quantile normalization and background corrected) until the most recent update on Sep. 05, 2008. After calculation the average expression values for duplicated samples, finally 243 tumor tissue samples, 10 normal tissues and 1 cell line sample from GBMs patients were used for differential expression analysis. The microRNA expression experiments were performed on Agilent 8 × 15 K Human miRNA-specific microarrays by researchers at University of North Carolina, NC, USA. There are 534 miRNAs (470 human miRNAs) and 240 tumor tissue samples, 10 normal tissue samples which are available in level three data (after quantile normalization and batch adjusted) until the most recent update on Nov. 10, 2008. Since, it is very difficult to get the brain tissue samples from normal people, all the control samples were from the adjacent normal tissues of GBMs patients. Thus, to detect the effect of somatic difference on disease was focused, which was also a common approach in other cancer studies. The 254 samples were used for gene expression and pathway analysis, 250 samples for microRNA expression analysis, 247 samples common in microRNA and gene expression datasets for microRNA targets analysis. The gene expression analysis was done[5]. A total of 1,236 genes were identified to be significantly differentially expressed between tumor and normal tissues. To further investigate the function of these differentially expressed genes, DAVID method was used. The bioinformatics resources and pathway analysis for systematic and integrative analysis of large gene lists was performed. 1,221 of 1,236 differentially expressed genes had annotations in DAVID Functional Annotation Tools. The gene set enrichment analysis were carried out to identify the most enriched gene function annotation terms (GO terms) in the list of 1,221 annotated differentially expressed genes. The top 10 enriched GO terms in the list of differentially expressed genes showed that these genes were enriched in brain and mainly associated with development and function of nervous system. DAVID could cluster similar functional GO terms together. The first two enriched GO term groups in the differentially expressed gene list were all the functional terms relevant to brain and neurons. These were, 1, GOTERM Cellular Component including 5 terms: neuron projection, cell projection, dendrite, cell soma, and axon. All 53 genes belonged to this cluster including CDK5, SNCG, UCHL1 and FREQ for cancer. The pathway analysis was also performed by using algorithm as proposed in TAPPA (Topological Analysis of Pathway Phenotype Association). The results revealed that 131 pathways were significantly associated with GBMs. The 131 associated pathways belonged to 33 functional groups, among which Cell signaling, Neuroscience, Immunology and Expression were the most enriched pathway groups. Glioma pathway was the only significant pathway in the cancer functional group with p-value = 5.75×10^{-7}. Similar to the GO terms enrichment analysis, the DAVID Functional Anno-

tation Tools were used to identify which pathways were most enriched in the list of differentially expressed genes and 40 significant pathways were found. Cell signaling, signal transduction, apoptosis and neuroscience were the most enriched pathway groups. The long-term potentiation (nervous system pathway) and Calcium signaling pathway (a signal transduction pathway), were the most significantly enriched pathways with p-value 2.62×10^{-8} and 3.26×10^{-8}, respectively. There were 11 significant cell signaling pathways, 4 significant apoptosis pathways, 4 significant signal transduction pathways, 3 significant immunology pathways, 3 significant neuroscience pathways and 2 significant nervous system pathways (some pathways may belong to different functional groups). The results suggested that the differentially expressed genes were most involved in signal, apoptosis and neuroscience pathways.

The analysis of differential expression of microRNA was done. A total of 97 microRNAs were significantly differentially expressed between tumor and normal tissues. To examine whether these miRNAs were associated with GBM, the miR2Disease was used to validate the results (Updated Date: Dec. 19, 2008). The miR2Disease provides a comprehensive literature reported resource of miRNA deregulation in various human diseases. From the data in miR2-Disease, 81 of the 97 significant miRNAs have been reported to associate with 84 diseases, among them 72 miRNAs are associated with 59 cancers and brain diseases. The 22 of those miRNAs have been reported to induce glioblastoma or glioblastoma multiforme (GBM)/Neuroblastoma (NB) and the expression pattern of miRNA in the literature is the same as that in data. The p-values were also shown for the 22 miRNAs. It was found that the other 50 miRNAs which were related to other cancers and brain diseases may also be important for carcinogenesis in brain but needs further confirmation. Among 97 significant miRNAs, 30 of those were up-regulated and 67 were down-regulated. To examine the function of those significant miRNAs, experiments and the regression analysis for miRNA and gene expression were carried out to find out the target gene of miRNAs associated with GBM.

Regulation of microRNAs

The regulation of gene expression by microRNA was determined. The miRNA promotes degradation of target mRNA or suppresses translation of corresponding protein by matching with mRNA in the 3'-UTR region. The miRNAs perform various biological functions through regulation of gene expression. To find out how miRNA regulates gene expression in GBM, the target genes of miRNAs were identified and constructed miRNA target networks. Since miRNAs repress the expression of its target gene, the first step was to test the inverse relationship between the expression profile of miRNA and its potential targets. The expression of target mRNA on the expression of miRNAs and select mRNA with significant negative regression coefficients as miRNA targets was seen. The p-value for declaring significant evidence of miRNA target was 1.00×10^{-4}. The second step was to conduct sequence analysis which used sequence complementarities of miRNA and its target site to predict potential miRNA target genes. This was done by using experimentally verified and predicted miRNA targets data from three miRNAs databases: miR-2Disease, TarBase and miRBase. The miR2Disease (updated on Dec.19, 2008) and TarBase (updated on June, 2008) provided experimentally verified miRNA target genes. The miRBase predicted the target gene of miRNA by miRanda algorithm, where the predicted target genes and miRNAs could be downloaded directly (updated on: Oct. 31, 2007) (Dong et al, 2010)[5]. The 1,236 differentially expressed mRNAs and 97 differentially expressed miRNAs data in 237 tumor tissue sample and 10 normal tissue samples were compiled. Two experimentally confirmed results were found. It is reported in the literature that in nasopharyngeal carcinomas underexpressed hsa-mir-29c (expression fold change (tumor/normal) = 0.20) target over expressed gene COL 4A1 (expression fold change (tumor/normal) = 5.24) and down-regulated hsa-mir-29c (differentially expressed p-value $< 5.11 \times 10^{-12}$) targets over-expressed gene COL4A1 (differentially expressed p-value $< 3.58 \times 10^{-6}$) with regression $\beta = -389.02$ and $p = 1.35 \times 10^{-8}$ were found. In conclusion, hsa-miR-29c is also an important miRNA in glioblastomas.

Another experiment validated that targets gene was LDOC1 targeted by has-miR-155. The known oncogenic miRNA hsa-miR-155 can regulate a set of target genes including LDOC, a regulator of apoptosis. The results showed that hsa-miR-155 was over-expressed (differentially expressed p-value $< 1.40 \times 10^{-10}$) and targets under-expressed gene LDOC1 (differentially expressed p-value $< 1.085 \times 10^{-31}$) with regression

$\beta = -196.77$ and $p = 4.00 \times 10^{-15}$. It was inferred that hsa-miR-155 could induce cancer through regulation of apoptosis gene LDOC1 in glioblastomas. For predicted targets in miRBase, 1,094 matched miRNA-gene pairs including 70 miRNAs and 661 genes were observed. The 44 down-regulated miRNAs target 202 over expressed genes while 26 up-regulated microRNAs target 459 under expressed genes. The 661 target genes were a subset of the 1236 significant differentially expressed genes. The pathways were examined for these genes enriched in and compared these with the previous results. The 11 pathways were significant by fisher exact test in DAVID, 8 of which were the same as the pathways identified from the previous sections. The statistical analyses were performed. The differential expression of the gene and microRNA were tested by **t**-test and Mann-Whitney Test. The thresholds for declaring significance after Bonferroni correction for multiple tests were 4.15×10^{-6} and 9.36×10^{-5}, for gene and miRNA respectively. Linear regression was used to investigate the relationships between miRNA and gene expressions. The linear model took its common form: where y is an n-by-1 vector of observations, such as gene expression and X is an n-by-p matrix of regressors, such as miRNA expression, β is a p-by-1 vector of parameters; known as regression coefficient and ε is an n-by-1 vector of random disturbances. The Right-tail Fisher exact tests were used to examine for the enriched Gene Ontology Terms, pathways in the datasets.

miRNA Expression in Glycolysis and Glioma

Recent studies by Wong et al[6] observed that altered energy metabolism is wide-spread in cancer cells along with other associated cells as hallmarks of cancer. The Akt signaling pathway is involved in the aerobic glycolysis program. However, mechanisms underlying the regulation of aerobic glycolysis and Akt activity in gliomas remain unclear. The microRNAs are a group of small non-coding RNAs that can function as endogenous RNA interference to regulate expression of targeted genes. This study was conducted to detect the function of miR-7 targeting insulin-like growth factor 1 receptor (IGF-1R), which is an upstream regulator of Akt. The microRNA expression data for GBM and normal controls were downloaded from The Cancer Genome Atlas (TCGA) database. Quantitative real-time PCR was used to measure the micro-RNA-7 (miR-7) expression level, and Western blot was performed to detect protein expression in U87 and U251 cells. Colony formation assay and glycolysis stress test were also performed. The Luciferase reporter assay was used to identify the mechanism of IGF-1R and miR-7 regulation. The miR-7 was down regulated in human glioma tissues based on TCGA database. The forced expression of miR-7 or IGF-1R knockdown inhibited colony formation and glucose metabolic capabilities of glioma cells *in vitro* and decreased the p-Akt expression. The Bioinformatics analysis results indicated that IGF-1R could be a target of miR-7. Western blot and Luciferase reporter assays showed that miR-7 modulated IGF-1R expression by directly targeting the binding site within the 3'-untranslated region. This study provides the first evidence that miR-7 inhibited cellular growth and glucose metabolism in gliomas at least partially, by regulating the IGF-1R/Akt signaling pathway. Therefore, miR-7 is a promising molecular drug for glioma treatment. The biological features of GBM offer opportunities for the design of a new therapeutic strategy based on targeting essential signaling pathways.

Gabriely's project[7] proposes to reprogram the monocytes and restore their intrinsic ability to destroy abnormal tumor cells. Recently, her team used microRNAs and discovered master genes to activate monocytes. Initially, to identify potential microRNA targets, the team compared their content between normal blood monocytes and glioma-associated monocytes. Therapeutic microRNAs were then identified by using a screen system of co-culturing monocytes with glioma cells. Therapeutic properties of microRNA modulation in monocytes invading glioma cells in an established mouse glioma model were validated. This pioneering research is promising to rescue glioblastoma patients from developing a new way to treat this devastating brain malignancy. It will provide the fundamental scientific information about immune cells populating glioma for comprehensive understanding of glioma, brain cancer biology which is necessary for the development of targeted treatments.

Neoplasms of CNS and Glioblastoma

Somasundaram et al[8] reported neoplasms, arising in cells originating from central nervous system (CNS) or peripheral nervous system (PNS) result in a class of tumors called as neuroectodermal tumors. Generally after development ceases, neurons become post-mitotic and only the glial

cells retain the capacity to divide and proliferate. Most of the brain tumors are of glial origin and are referred as gliomas. Based on the cell type involved in malignancy, gliomas can be astrocytomas (astrocytes), oligodendrogliomas (oligodendrocytes), oligo-astrocytomas (mixture of astrocytes and oligodendrocytes) and ependymomas (ependymal cells). Astrocytomas account for more than 60% of all primary brain tumors, hence forming the majority of neoplasms of the CNS. The neurofibroma and schwannoma are the two most common glial tumors of the PNS. The World Health Organization[9] classification showed, Astrocytomas are classified into grades I-IV based on the intensity of malignancy as determined by histopathological criteria. The Grade I (Pilocytic Astrocytoma; PA) gliomas are generally benign and often circumscribed, whereas grade II (Diffuse astrocytoma; DA), grade III (Anaplastic Astrocytoma; AA) and IV (Glioblastoma; GBM) gliomas are malignant and diffusely infiltrate the brain. The most malignant and common forms of infiltrating astrocytic tumors are glioblastoma with a median survival of less than one year.

The GBM can be further divided into two subtypes based on certain genetic and clinical features: as primary and secondary GBM. Primary GBM arises as a *de novo* process, without any antecedent history of low-grade lesions, whereas secondary GBM develops progressively from lower grades, generally over a period of 5-10 years. The underlying cause of gliomagenesis is the genomic alterations like deletions, amplifications, mutations, aberrant DNA methylation and chromosomal rearrangements, which results in the activation of oncogenes and inactivation of tumor suppressors. The hundreds of miRNA genes are discovered in the past few years that produced non-coding RNA transcripts with no significant open reading frame. It has become evident that the genomic complexity of the cancer cell is far greater than expected. The miRNAs are single-stranded non-coding RNAs of 19-25 nucleotides in length, generated from endogenous transcripts that contain a local hairpin structure. These function as guide for cancer.

The molecular targets of CNS tumors 344molecules in post-transcriptional gene silencing by base pairing with target mRNAs, leading either to the cleavage of the target mRNA or its translational repression. The miRNAs are one of the largest gene families, accounting for ~1% of the genome[10]. Recent studies suggest that the miRNAs play a key role in diverse regulatory pathways which control development, differentiation, apoptosis, cell proliferation, and organogenesis. The miRNAs and their targets seem to form intricate regulatory networks, as single miRNA can bind to and conversely regulate different mRNA targets. A single mRNA can be synergistically controlled by multiple miRNAs. Recently, Bartel and Burge[11] predicted that over one third of the human genes are regulated by miRNAs. The location of miRNA genes in fragile sites of the genome provides a circumstantial evidence of an etiological role of miRNAs in tumor formation. The miRNAs are generally negative regulators of gene expression. The changes in the amounts of these RNAs can be tumorigenic if these target a tumor suppressor or an oncogene. For example, over accumulation of a miRNA that targets a tumor suppressor would result in less of that protective factor. The reduced accumulation of a miRNA that targets a proto oncogene could lead to excessive amounts of the oncogenic protein. The net outcome is an imbalance in the activities of tumor suppressor genes and oncogenes, hence results in malignancy.

The detection and biology of miRNAs was reviewed including their structure, regulation, and biogenesis along with different methods to assay miRNA expression. A detailed account of the literature on differential miRNA expression and their functional characterization in glioma is provided. The potential utility of miRNAs in glioma diagnosis, classification, prognosis, and targeted therapy is also described.

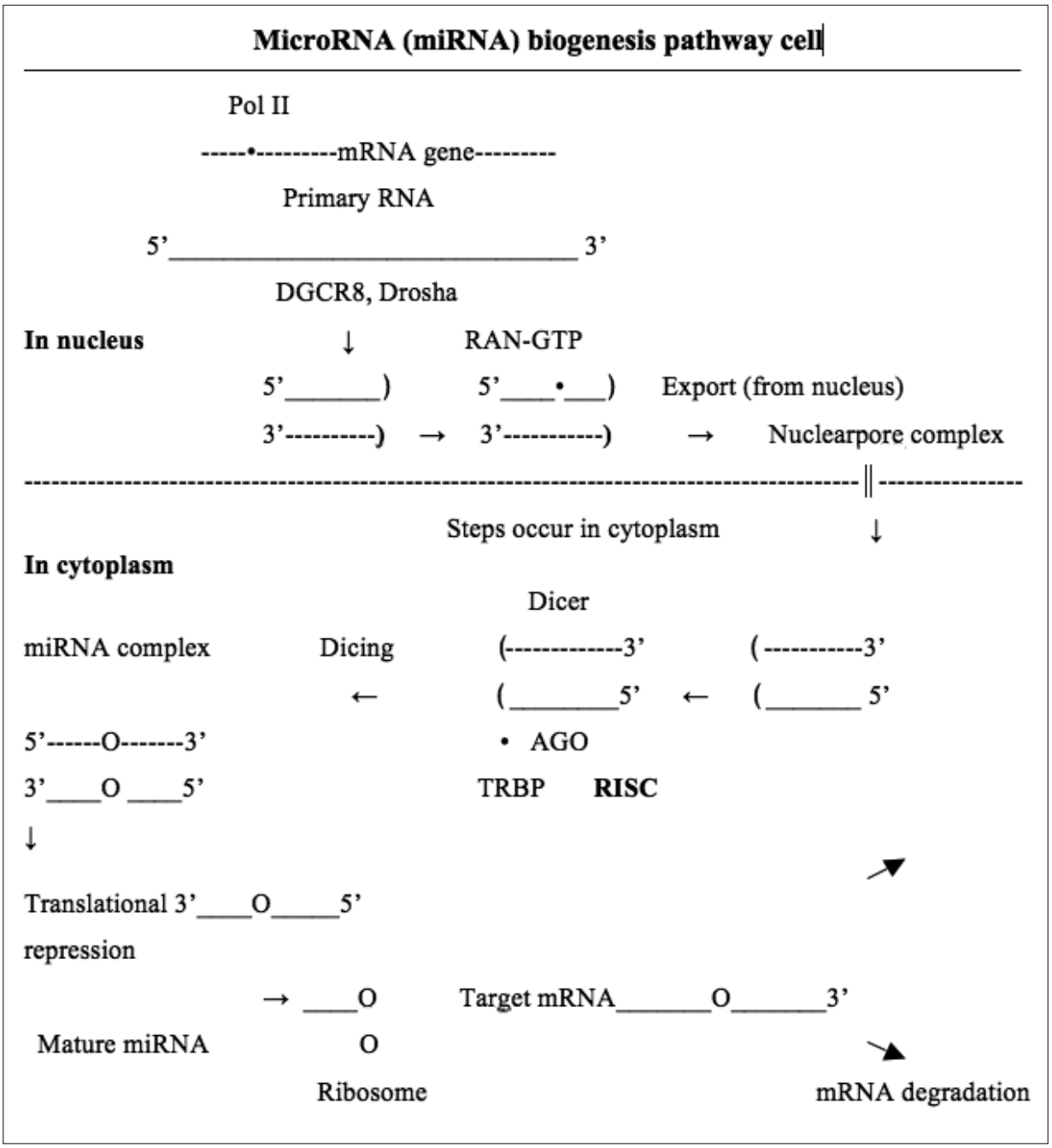
Detection and Biology of MicroRNAs

The microRNAs were initially discovered as small temporal RNAs (stRNAs) that played a role in the developmental transitions in *Caenorhabditis elegans*. The first miRNA was discovered as lin-4 in *C. elegans* in 1993[12]. The discovery of lin-4 and its target specific translational repression pointed to a new way of gene regulation. In 2000, the second miRNA, let-7 was discovered in the same organism and since then many miRNAs have been discovered. A total of 15172 miRNA have been recorded from various organisms (Release 16, miRBase) which also include 1048 miRNAs from Homosapiens (http://www.mirbase.org/). A small RNA must fulfill the following criteria to be classified as miRNA. Its expression should be authenticated by hybridization to a size-fractionated RNA sample by employing Northern blotting. It should be

detected by methods like PCR after reverse transcription of RNA (RT-PCR), primer extension analysis, RNase protection assay and microarray. However, Northern blotting remains the method of choice for the confirmation of miRNAs, as the blot generally detects both the mature form (a ~22-nucleotide band) and the hair pin precursor (a ~70-nucleotide band) of the predicted miRNA. The small RNA sequence must exist in the stem portion of the hairpin precursor. The precursors are ~60-80 nucleotides in animals, but the lengths are more variable in plants. The small RNA sequences should be conserved phylogenetically. The evidence can be consolidated if the precursor of the predicted miRNA accumulates in a blated Dicer background. This criterion is not in vogue, because of the technical challenges that are involved in depleting Dicer in different cells. The miRNA gene structure showed that most miRNA genes are transcribed as independent transcription units. The transcripts thus synthesized are referred as primary miRNAs and are equipped with a 7-methyl guanosine cap in the 5' end as well as a poly A tail in the 3' end which are the unique features of class II gene transcripts. The miRNAs arising from a single gene cluster can be related to each other, suggesting that the miRNAs might have assembled as a result of gene duplication and may play a synergistic role in the production of a particular phenotype. The intragenic class can be either intronic or exonic in nature owing to the fact whether the stem loop sequence of the precursor miRNA comes from the intron or exon of the protein coding gene or sometimes a non-protein coding gene. In both types, the miRNA gene can be mono- or polycistronic. The expression of intragenic miRNAs can be controlled by their own independent promoter or the miRNAs would be co-transcribed with the parent gene, in which case the pre-miRNA would be processed to intron splicing. Some miRNAs derive their stem loop sequence from short introns and hence bypass the processing action of Drosha and are called as Mirtrons. The miRNA gene regulation and expression can be modulated at a number of levels, which include the transcription of the primary transcript and the multiple steps of the miRNA biogenesis. The diagrammatic representation of miRNA biogenesis pathway in cell is described in Somasundaram et al[8]

The cell has nucleus and cytoplasm compartments separated by nucleopore membrane complex. In nucleus, the miRNAs co-transcribed with the parent gene, pre-miRNA and processed to intron splicing. In the cytoplasm, after dicer formation and dicing, the miRNA complex is formed. This makes a mature miRNA and then ribosome records the message on target mRNA strand. This makes either translation repression or mRNA degradation.

A number of RNA polymerase II associated transcription factors regulate the expression of miRNA genes, for instance TP53; a tumour suppressor transcription factor activates the miR-34 family of miRNAs. The majority of miRNA genes being transcribed by the same RNA polymerase as protein coding genes, the mechanisms of epigenetic control known for protein coding genes are likely to be applicable to miRNA loci, the aberrant DNA methylation of tumor suppressor genes. The miRNA promoters are also regulated by histone modifications during the development and pathogenesis. The biogenesis of miRNA involves initial transcription as a largely unstructured precursor, termed as primary miRNA (pri-miRNA), which is subsequently processed in the nucleus to give rise to a 70-nucleotide precursor miRNA hairpin intermediate (pre-miRNA). The pre-miRNA gets transported to the cytoplasm where it undergoes further processing to give rise to a mature miRNA. Most of the miRNA genes are transcribed by RNA polymerase II to generate a stem-loop containing primary miRNA (pri-miRNA), which can range in size from hundreds of nucleotides to tens of kilobases. Like mRNAs, RNA polymerase II transcribed pri-miRNAs contain 5' cap structures are polyadenylated and may be spliced. The pri-miRNA is processed within the nucleus by a multiprotein complex called the microprocessor. The microprocessor is a 650 kDa complex in humans and is constituted by two key components: Drosha, an RNase III enzyme, and DiGeorge syndrome critical region 8 gene (DGCR8), a double-stranded RNA-binding domain (dsRBD) protein. For miRNA regulation in glioma, several studies have in depth reported the specific miRNAs in glioma cells including functional characterization to understand the effects of regulation of miRNA in glioma cells with respect to various transformation related phenotypes like cell proliferation, cell death, migration and invasion. For large scale expression profiling of miRNAs in glioma the microarray chip was used to profile the expression of 245 miRNAs in GBM tissue samples. The samples included 9 GBM tissue samples, matched adjacent peripheral region as



normal brain tissue and glioma cell lines. With the additional validation by Northern blotting, this study identified miR-221 as GBM up regulated and miR-128, miR-181a, miR-181b and miR-181c as GBM down regulated miRNAs, analyzed the expression of 180 mammalian miRNAs using a nylon membrane based oligo nucleotide array in 3 primary high-grade gliomas and 8 non-neoplastic fetal and adult brain tissues. The miR-21was found as the most up regulated miRNA in high-grade gliomas among a total 8 miRNAs. This was validated by the expression of selected miRNAs (miR-16, miR-17, miR-19a, miR-20a, miR-140 and miR-184) in an independent set of low grade and secondary GBM samples, thus, confirming the grade associated regulation of these miRNAs. The modulation of miR-17 and miR-328 in glioma cell lines showed an important role of these miRNAs in glioma cell proliferation, apoptosis and cell invasion.

Niyazi et al[13] studied new prognostic subgroups in patients with glioblastoma, GBM, a miRNA screen (> 1000 miRNAs) from paraffin tissues followed by a biomathematical analysis. The 35 glioblastoma patients were treated between 7/2005-8/2008 at a single institution with surgery and post-operative radio (chemo) therapy were included in this retrospective analysis. For microarray analysis the febit biochip "Geniom® Biochip MPEA homo sapiens" was used. The total RNA was isolated from FFPE tissue sections and 1100 different miRNAs were analyzed. It was possible to define a distinct miRNA expression pattern allowing for a separation of distinct prognostic subgroups. The defined miRNA pattern was significantly associated with early death versus long-term survival (split at 450 days) (p = 0.01). The pattern and the prognostic power were both independent of the MGMT status. This is the first dataset defining a prognostic role of miRNA expression patterns in patients with glioblastoma. This pattern was also defined by a prospective study of brain and meninges[14]. The classification of multiple cancers based on the expression pattern of couple of hundred miRNAs was found to be more accurate than using expression of approximately 16,000 protein-coding genes as reported earlier[15]. It suggests within a glioma grade that distinct subgroups within each grade of glioma could be identified.

Conclusions

miRNAs regulation has significance in glioma development and GBM pathogenesis. Its role in cell proliferation, apoptosis, cell cycle regulation, migration, invasion and angiogenesis has been suggested. This indicates that defects in miRNA regulatory network appear to play a key role in glioblastoma pathogenesis. With total number of known human miRNAs as 1040 (Release 16, miRBase), one of important needs is to carry out more comprehensive analysis of all known miRNAs in large number of tumor tissue samples. The efforts towards identifying targets, their experimental validation and establishing the relationships with various altered molecular pathways in cancer are also very important. The miRNA provides new useful method for clinical research. Similar to other biomarkers, the use of miRNAs in glioma classification and prognosis requires more studies in large cohorts at independent labs. The use of miRNA based glioma therapy is very promising. The development of better methods of *in vivo* delivery of miRNAs is likely to show the utility of miRNA in glioma, GBM brain cancer therapy.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Costa PM, Cardoso AL, Mano M, de Lima MC. US National Library of Medicine National Institutes of Health. MicroRNAs in glioblastoma: role in pathogenesis and opportunities for targeted therapies. CNS Neurol Disord Drug Targets 2015; 14: 222-38.
2) Novakova J, Slaby O, Vyzula R, Michalek J. MicroRNA involvement in glioblastoma pathogenesis. Mini Review. Biochem Biophys Res Commun 2009; 386: 1-5.
3) American Brain Tumor Association 2014. Glioblastoma and malignant astrocytoma. www.abta.org Retrieved 4 September 2014.
4) Silber J, Lim DA, Petritsch C, Persson AI, Maunakea AK, Yu M, Vandenberg SR, Ginzinger DG, James CD, Costello JF, Bergers G, Weiss WA, Alvarez-Buylla A, Hodgson JG. miR-124 and miR-137 inhibit proliferation of glioblastoma multiforme cells and induce differentiation of brain tumor stem cells. BMC Med 2008; 6: 14.
5) Dong H, Siu H, Luo L, Fang X, Jin L, Xiong M. Investigation gene and microRNA expression in glioblastoma. BMC Genomics 2010; 11(Suppl 3): S16.
6) Wang B, Sun F, Dong N, Sun Z, Diao Y, Zheng C, Sun J, Yang Y, Jiang D. MicroRNA-7 directly targets insulin-like growth factor 1 receptor to inhibit cellular growth and glucose metabolism in gliomas. Diagn Pathol 2014; 9: 211.
7) Gabriely G. The role of microRNAs monocyte-mediated glioma pathogenesis. Brain Science Foundation 2015 (Internet).
8) Somasundaram K, Alige S, Raoand M, Nawaz Z. MicroRNA (miRNA) regulation in glioma: implications in development, progression, grading, prognosis and therapy. Ind Inst Sci, Bangalore, India. CNS Tumors 2011 www.intechopen.com.
9) WHO. World Health Organization, Geneva, Switzerland, Report on brain cancer. 2007.
10) Kim VN. MicroRNA biogenesis: coordinated cropping and dicing. Nat Rev Mol Cell Biol 2005; 6: 376-385.

11) Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005; 120: 15-20.

12) Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell: 1993; 75: 843-854.

13) Niyazi M, Zehentmayr F, Niemöller OM, Eigenbrod S, Kretzschmar H, Schulze-Osthoff K, Tonn JC, Atkinson M, Mörtl S, Belka C. MiRNA expression patterns predict survival in glioblastoma. Radiat Oncol 2011; 6: 153.

14) Smirniotopoulos JG, Murphy FM, Rushing EJ, Rees JH, Schroeder JW. From the Patterns of contrast enhancement in the brain and meninges. Radiographics 2007; 27: 525-551.

15) Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834-838.