



THE NOVEL CIRCULATING BIOMARKERS OF GLIOMAS- A REVIEW

Priavadhana Rajan Prasaad¹, Krishna Prasanth.B²,Vindu Srivastava^{3*}

¹ Assistant Professor of Pathology, ESIC Medical College and PGIMSR, Chennai.

²Epidemiologist & Assistant Professor of Community Medicine,Sree Balaji Medical College and Hospital, Bharath Institute of Higher Education & Research, Chennai.

³Professor of Pathology,Sree Balaji Medical College and Hospital, Bharath Institute of Higher Education & Research, Chennai

*Corresponding author-Dr.Vindu Srivastava MD (Pathology),
Professor of PathologySree Balaji Medical college and Hospital, #07 Works road ,
Chrompet,Chennai-600044,TN, India

ABSTRACT

Biomarkers are substances that are helpful for both diagnostic and prognostic purposes. Since diffuse infiltrating gliomas are difficult to treat, an earlier diagnosis using these biomarkers provide an earlier non-invasive method of diagnosis with an increase in survival rate. Several circulating tumor markers are available for gliomas which can be detected in blood as well as cerebrospinal fluid. A biomarker for a neoplasm could be a tumor cell, a normally or abnormally elaborated protein, nucleic acids or an enzyme specific to the cell of origin. In gliomas, these range from circulating tumor cells (CTCs), circulating tumor derived exosomes (CTdE), circulating tumor associated Nucleic acids (CNAs), circulating tumor associated proteins (CTaP) and biomarkers produced by tumor associated inflammatory cells. This review focusses on the various available biomarkers which serve as prognostic indicators in diffuse gliomas and glioblastomas

KEYWORDS: Glioma, Biomarkers, Tumor

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INTRODUCTION:

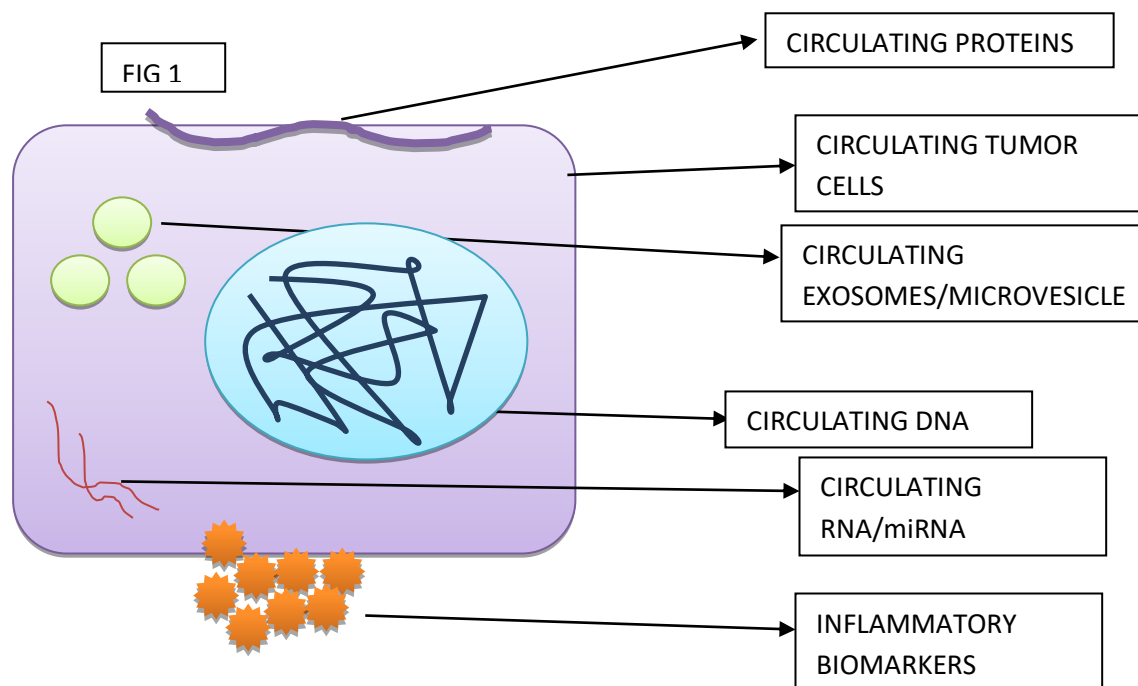
Biomarkers are substances that are helpful for both diagnostic and prognostic purposes. Since diffuse infiltrating gliomas are difficult to treat, an earlier diagnosis using these biomarkers provide an earlier non-invasive method of diagnosis with an increase in survival rate. Several circulating tumor markers are available for gliomas which can be detected in blood as well as cerebrospinal fluid. This review aims to give an insight into the various available biomarkers for gliomas.

UNDERSTANDING THE BIOMARKER COMPONENTS:

A biomarker for a neoplasm could be a tumor cell, a normally or abnormally elaborated protein, nucleic acids or an enzyme specific to the cell of origin. In gliomas, these range from circulating tumor cells (CTCs), circulating tumor derived exosomes (CTdE), circulating tumor associated Nucleic acids (CNAs), circulating tumor associated proteins (CTaP) and biomarkers produced by tumor associated inflammatory cells.



FIG 1: DIAGRAMMATIC REPRESENTATION OF POTENTIAL BIOMARKERS:



CIRCULATING TUMOR CELLS:

Circulating tumor cells have recently gained significance in metastatic disease in that they help in the prediction of disease free survival rates and overall progression of disease. But, only few cases of diffuse infiltrating gliomas and glioblastomas metastasize to distant sites and hence the detection rate is very less compared to solid tumors in other systems. Only one cell in 10^9 cells will be a circulating tumor cell and hence robust detection methods to be employed for the identification of these CTCs.

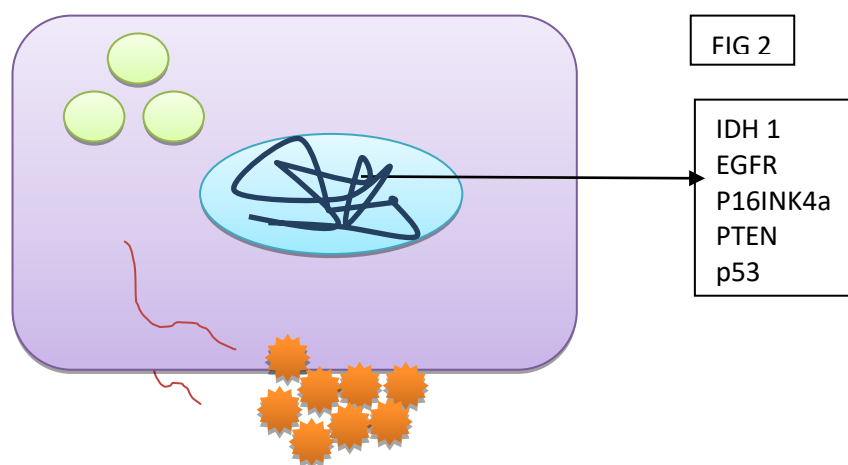
CIRCULATING NUCLEIC ACIDS:

The circulating tumor specific nucleic acids are biomarkers which can be detected by high throughput PCR assays and hence are

promising biomarkers for the early detection of distant spread. But, since the quantity of circulating nucleic acids are of negligible amounts, it still poses a difficulty in detection. These circulating nucleic acids can be a part of tumor DNA or RNA or microRNA. CNAs which have abnormal tumor DNA in circulation, may express the signature mutations seen in gliomas. The most commonly seen CNAs are IDH1 mutation which play a major role in the early gliomagenesis and also in majority of secondary glioblastomas. Other cDNA which can be detected as biomarkers for primary glioblastomas are EGFR, p16Ink4a, PTEN and p53 gene products. These tumor markers are the major molecular gene alternations seen in primary glioblastomas.



Fig 2: Illustrates the potential nucleic acid markers in glioblastomas.



CIRCULATING RNA AND microRNA:

ctRNAs are relatively difficult to detect in the serum of glioma patients since RNAs are unstable and are easily degraded by the ribonucleases which are present in abundance in any cell and in the serum. IDH 1 RNA and EGFRvIII RNA are the two glioma associated tumor markers which can be detected in the serum of the patients.

MicroRNAs (miRNAs) which are gene regulatory molecules composed of 22 nucleotides make a promising group of tumor markers in gliomas. These are relatively stable and hence can be detected in the serum and the cells of patients. Some of the miRNAs which can be potential biomarkers for gliomagenesis are miR15b, miR23a, miR133a, miR150, miR197, miR497 which can be detected in the serum of glioma patients. These can be detected by PCR or gene sequencing techniques.

CIRCULATING PROTEIN BIOMARKERS:

Since glioblastomas are very vascular tumors, the proteins associated with vasculogenesis and neoangiogenesis in glioblastomas could be used as potential biomarkers. Proteins implicated in the pathogenesis of gliomas can form this group of serum biomarkers which could be detected by ELISA. The major group of proteins implicated in vasculogenesis are VEGF, VEGFR, PDGF, Angiopoietin and few others. Also, certain matrix proteins which are secreted by the tumor cells into the

extracellular tumor environment or in circulation could be detected using ELISA. The matrix associated proteins which can be detected are Tenascin, Osteopontin, SPARC (secreted protein acidic and rich in cysteine), thrombospondin, few Matrix metalloproteinases and many more. Glial fibrillary acid protein which can be detected using immunohistochemistry in tissue sections are highly elevated in serum of glioblastoma patients, and hence can be used as a protein biomarker in these patients.

EPIGENETIC BIOMARKERS IN GLIOMAS:

Gliomas are associated with a profound reduction in T cell immunity. Various reviews states that when estimated, the peripheral blood T cell counts are reduced in number in many glioma patients. The quantification of Tregs (T cell regulators) serve as a prognostic epigenetic biomarker in gliomas. Though the counts of CD3 and CD4 cells are also decreased in gliomas patients, the ratio of Tregs to that of T cells serve as a better prognostic indicator. It is found in some studies that, the higher ratio correlates with a poor prognosis. Specific markers for regulatory T cells like FOXP3 could serve as epigenetic biomarkers for these patients.

IMMUNE BIOMARKERS:

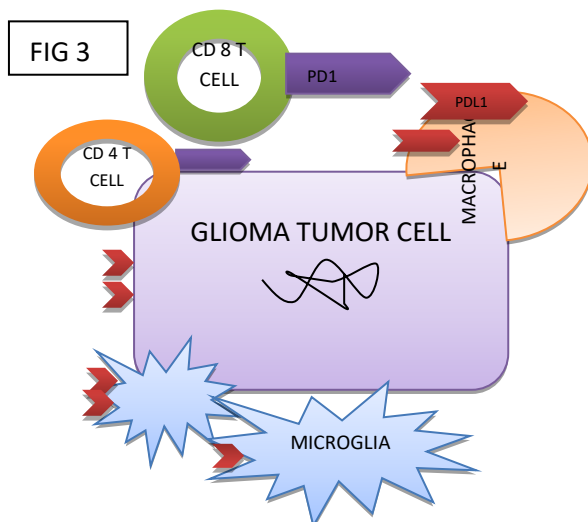
The molecular crosstalk between cancer cells and immune cells is well established. In glioma microenvironment, the tumor infiltrating immune cells serve as a prognostic



marker for predicting the disease free survival rates in these patients. It has been found that Programmed cell death Ligand 1 (PDL1) and Programmed cell death 1 (PD1) are increased in the tumor milieu and these can be used as strong poor prognostic indicators in glioblastomas. The role of PDL1 in helping the cancer cells evade host immunity is well

established in tumors of lung and melanomas. Many studies have shown that there is an increased expression of PDL1 and its ligand PDL-1 in the tumor microenvironment. There are experimental studies which have shown that these immune markers are also seen in circulating tumor cells and hence, can be used as an effective biomarker in glioblastomas.

Fig 3: Projected model of PD1 and PDL1 in tumor microenvironment and its role in evasion of tumor immunity



As depicted in the above image, there is an increased expression of PDL1 not only on the glioma tumor cells but also on the microglia and the immune cells including the macrophages and the circulating monocytes. Hence, it can serve as an effective biomarker for the detection of immune response state of a patient with glioma. Targeted therapy with monoclonal antibodies are available to inhibit the expression of PDL1 and hence the binding of PD1 with its ligand which enhances the host cell immune response resulting in tumor cell lysis.

MISCELLANEOUS MARKERS:

Chitinase 3 like 1 is a gene which regulates many important steps of cell differentiation, apoptosis, proliferation and tissue remodelling. The end product of the gene, found in circulation is YKL-40 which is a glycoprotein which can be detected by using RTPCR and other methods. It has been observed in few studies, that this end product of the gene could serve as a biomarker in predicting the prognosis in few tumors including glioblastomas. In a few studies, a

strong correlation between the levels of YKL 40 and grade of gliomas was noted. It has been observed that CHI3L1 levels are altered in the earlier stages of glioma and there was a significant increased expression in the advanced stages of gliomas. CHI3L1 gene plays a role in the inflammatory response mediated by Th2 T cells and also participate in IL 13 mediated inflammation. Also, this gene promotes apoptosis, macrophage differentiation and regulates hyperoxia induced injury. The pathways of glioma pathogenesis and CHI3L1 is not very clear but could be due to the altered immunogenic response, which helps the tumor cells to evade the innate immune mechanisms. Also, hypoxia induced injury and activation of Glioma stem cells are known as the earlier steps in the pathogenesis of glioblastomas. Hence, this gene could be implicated in the hypoxia mediated injury and hence an earlier hit in gliomagenesis.

Tumor associated antigens (TAA) as biomarkers in gliomas:



Tumor associated antigens have long been used as serum biomarkers for detection of many tumors like hepatocellular carcinomas, testicular tumors and colonic cancers. Few studies have been done on the TAAs expressed in gliomas. It has been found that there is a significant differential expression of genes like IGHG1, EYA1, SNX1 AND PQBP1 in the different grades of gliomas. SNX1 gene has been found to be associated with increase expression of EGFR in few studies. Hence, these gene markers and their associated proteins could serve as potential biomarkers in the future.

CONCLUSION:

The review focusses on the various available biomarkers which serve as prognostic indicators in diffuse gliomas and glioblastomas. The potential biomarkers can be a circulating tumor cell, circulating nucleic acid, circulating proteins expressed by the tumors cells or it could be an immune biomarker. Further experimental studies are needed to understand the newer biomarkers.

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