Putative Role for *METTL1* in Human Glioblastoma

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Abstract:

Glioblastoma multiforme (GBM) is a deadly cancer affecting the late adult and elderly population. Although GBM can be subdivided into a number of distinct types, aberrant methylation effects define certain subtypes, such as the glioblastoma CpG island methylator phenotype (G-CIMP) subtype, and more recently, the proneural subtype. In GBM as a whole, gene amplification of methyltransferases – enzymes that catalyze the transfer of a -CH3 methyl group to nucleic acids - are a common theme. This type of gene amplification event leads to hyper-activity of the enzyme, and contributes to the methylator phenotype; however, the role that the methylator phenotype plays in GBM not completely understood. Here we mined cancer genome datasets for alterations of methyltransferases that target the nucleic acid, transfer RNA (tRNA). *METTL1* was identified as the highest amplified tRNA methyltransferase in glioblastomas. *METTL1's* orthologue in yeast, TRM8, has been studied to some extent, and is known to target several tRNA species ($tRNA^{VAL\text{-}AAC}$, $tRNA^{MET}$, $tRNA^{PHE}$, $tRNA^{VAL}$) as part of the cell's response to heat shock (REF). Overall, this work suggests a putative role for tRNA methylation in GBM formation through the amplification of *METTL1*, and suggests that tRNA methylation is an important component of the methylator phenotype. Future work will be to identify which tRNAs are targeted by METTL1 and the role it plays in the development of GBM.

Introduction:

Glioblastoma multiforme (GBM) affects around 3 in 100,000 adults, manifesting around the age of 64 and affecting more Caucasian men than women or any other ethnicity. GBMs are the most common malignancies of the CNS and brain, and primarily present as grade 4 astrocytomas. Gliomas can affect the oligodendrocytes or the astrocytes at different grades. Stages 1 and 2 are low grade and are not as aggressive as stages 3 or 4 which grow very rapidly when compared to lower stages. Overall, GBM has a low survival estimate, and the prognosis is poor with an average of 2.5 years of survival following diagnosis.[1] This statistic underlies the importance of research aimed at understanding how this cancer develops, in order to discover innovative treatment strategies. [1]

Known GBM genetic alterations:

There are multiple subtypes of GBM including proneural, neural, classical, mesenchymal, and G-CIMP. Classical GBM tumors are a subtype that overexpresses epidermal growth factor receptor (EGFR), which is thought to drive proliferation. The tumor suppressor gene tumor protein p53 (TP53) is not mutated in this case, which may check some of the uncontrolled growth stimulated by EGFR, and patients with classical GBM have a longer survival than those with other GBM tumor subgroups.[2] GBMs 50% of all newly diagnosed GBMs involve methylated O(6)-methylguanine-DNA methyltransferase (MGMT) promoters [1], which has been suggested as a useful biomarker for the classical subgroup. Temozolomide (TMZ) is a new chemotherapeutic that alkylates guanine residues in DNA. These quanine residues cause extensive cell damage that leads to apoptosis. When MGMT, a DNA repair protein, is methlylated the TMZ does not function properly.. IIf the promoter for MGMT is methylated, the gene expression is activated in high levels that leads to chemotherapy resistance.

The mesenchymal subgroup shows an increased frequency in Neurofibromatosis type I (NF-1) tumor suppressor gene mutations as well as frequent mutations to TP53 and Phosphatase and tensin homolog (PTEN) suppressor genes. [2] Neural subgroups have mutations of tumor suppressor genes that are found in all subgroups, but additional have deregulated expression of genes typical to normal neurons. Patients with this tumor subgroup have a later onset, meaning they are older by the time the diagnosis is caught, and accordingly, have a slightly worse prognosis than those with classical or mesenchymal subgroups. [2]

Proneural tumors have a high frequency of mutated TP53, as well as mutations of the gene isocitrate dehydrogenase 1 gene (IDH1). IDH1 produces a protein that adds to the growth of abnormal cells. Another alteration seen only in proneural tumor subtypes involves the platelet derived growth factor receptor alpha (PDGFRA) gene and produces a protein that further stimulates uncontrolled growth.[2] Other genetic alternations, unique to proneural GBMs, give rise to the CpG island methylator phenotype (G-CIMP), which is associated with the highest amount of IDH1 mutations. The presence of G-CIMP are prevalent in low-grade gliomas, and patients with the phenotype can be diagnosed earlier than those with other subdivisions. [3,4]

Results

Figure 1: METTL1 with attention to Ser27 which has been modified

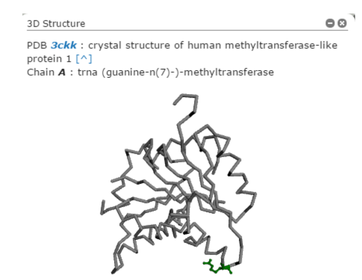

Figure 2: Gene amplification across central nervouse system cancers

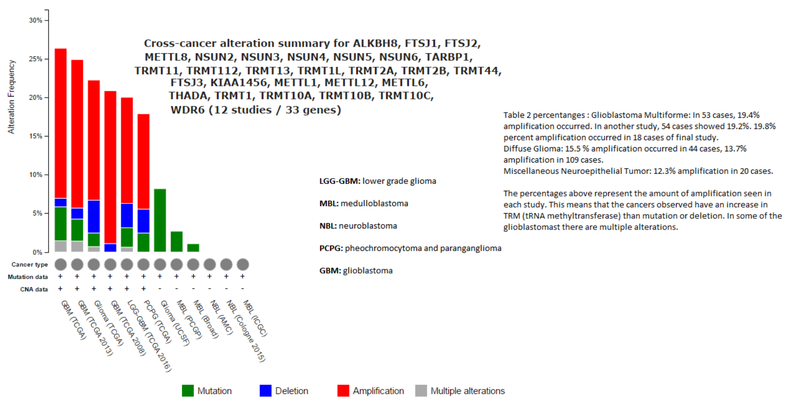

Table 2 percentanges : Glioblastoma Multiforme: In 53 cases, 19.4% amplification occurred. In another study, 54 cases showed 19.2%. 19.8% percent amplification occurred in 18 cases of final study.
Diffuse Glioma: 15.5 % amplification occurred in 44 cases, 13.7% amplification in 109 cases.
Miscellaneous Neuroepithelial Tumor: 12.3% amplification in 20 cases.

The percentages above represent the amount of amplification seen in each study. This means that the cancers observed have an increase in TRM (tRNA methyltransferase) than mutation or deletion. In some of the glioblastomast there are multiple alterations.

Figure 3:: METTL1 amplification

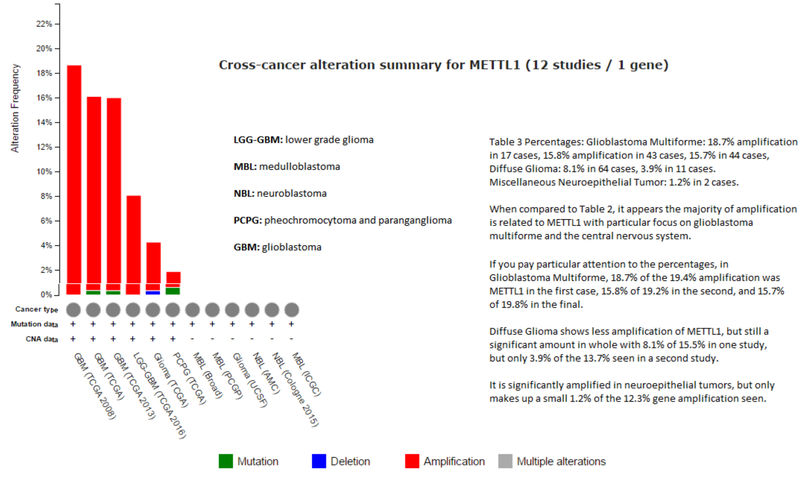

Table 3 Percentages: Glioblastoma Multiforme: 18.7% amplification in 17 cases, 15.8% amplification in 43 cases, 15.7% in 44 cases, Diffuse Glioma: 8.1% in 64 cases, 3.9% in 11 cases.
Miscellaneous Neuroepithelial Tumor: 1.2% in 2 cases.

When compared to Table 2, it appears the majority of amplification is related to METTL1 with particular focus on glioblastoma multiforme and the central nervous system.

If you pay particular attention to the percentages, in Glioblastoma Multiforme, 18.7% of the 19.4% amplification was METTL1 in the first case, 15.8% of 19.2% in the second, and 15.7% of 19.8% in the final.

Diffuse Glioma shows less amplification of METTL1, but still a significant amount in whole with 8.1% of 15.5% in one study, but only 3.9% of the 13.7% seen in a second study.

It is significantly amplified in neuroepithelial tumors, but only makes up a small 1.2% of the 12.3% gene amplification seen.

Results continued:

Literature search: METTL1 is a methyltransferase that is deactivated when phosphorylated by Protein Kinase B (PKB) and Ribosomal S6 Kinase (RSK) at serine 27. (SER27) in *S. cerevisiae*. It works with another protein called WDR4, TRM82 in yeast. *METTL1* is the homologue of S. cerevisiae *TRM8*, and is known to cause the m^7G modifications in most of the 11 yeast tRNA with that modification. This means it is likely to do the same in the 9 human tRNA that possess this modification. Furthermore, it is known that TRM8 provides an increased tolerance to heat. [5]

Conclusions:

In searching the cancer genome data base, we found that gene amplification of tRNA methyltransferases were frequent events in brain cancers, particularly glioblastomas (Figure 2). Thirty three genes corresponding to the human homologs of known yeast tRNA methyltransferases were searched across all central nervous system cancer studies. The main finding was that the gene encoding the METTL1 tRNA methyltransferase was amplified GBM. This suggests that increased METTL1 enzymatic activity plays a role in the development or survival of this type of brain cancer.

A repeat screen interrogating only METTL1 is shown in table 3, with similar studies emerging. Therefore, these two screens revealed that METTL1 is the primary human methyltransferase altered by gene amplification in GBM. To conclude, DNA sequence data indicates that the *METTL1* gene is amplified in a significant proportion (up to 20%) of GBM. Overall, this finding suggests a role for tRNA methylation in GBM development, and future studies will investigate the contribution of METTL1-mediated methylation to the phenotype of this type of cancers. It is possible that METTL1 may play a role in activities such as the methylation of MGMT promotors, although this would involve a DNA rather than RNA nucleic acid target for this enzyme.

References:

***www.cbioportal.org for all charts and graphs.

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