p53 level and MGMT promoter methylation are playing a role in Zika virus replication in glioblastoma

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Introduction
Glioblastoma (GBM) is a malignant primary brain cancer. It is a devastating cancer not only because of its poor prognosis, but also because it robs patients of their strength, memory, personality, and language. The poor median survival rate for patients with GBM of 15 months has not budged for the past 15 years, when the current standard treatment was first approved. There is no standard of care chemotherapy for recurrent GBM. Needless to say, novel treatments and treatment strategies for GBM are needed. One such novel treatment strategy is an oncolytic virus. Zika virus (ZIKV) affinity for fetal neural stem cells has made it a compelling candidate as an oncolytic therapy for glioblastoma. Previous studies have shown that ZIKV does infect and replicate in most but not all GBM cancer cells. Understanding the genetic milieu that is permissive for ZIKV infection is critical to the creation of a safe and effective viral oncolytic treatment. This report presents initial data for a ZIKV replication gene signature.

Methods
ZIKV strain MR766 was propagated in Vero cells. Viral stock was titrated by plaque assays. Western blot was used to characterize MGMT, AXL and p53 expression in eight commercial GBM cell lines (LN229, U87, A172, U251, LN18, T98G, U137, and U118). We have also used seven patient derived glioblastoma cancer stem cells to validate the hypothesis. The cell lines were stratified by MGMT promoter methylation status and were exposed to ZIKV at MOI 1. The percentage of infected cells was quantified by flow cytometry using the pan-flavivirus anti-E protein Ab 4G2.

Objective
The purpose of this study is to understand the role of p53 level and MGMT promoter methylation in ZIKV replication in glioblastoma cancer cells.

Results
ZIKV does not replicate in unmethylated-MGMT and elevated p53 expression glioblastoma cell lines.

Conclusion
Based on these results, there is a clear difference in the ability of ZIKV to replicate in GBM cell lines based on MGMT and p53 expression. Additional work is underway to understand the mechanism(s) underlying these findings and to define a ZIKV replication gene signature.

Future directions
• RNA-seq analysis of ZIKV replicating and non-replicating GBM cell lines to find differentially expressed genes.
• Validation of differentially expressed genes in ZIKV replication.

Fig 1. ZIKV infection and replication. (A and B) The human glioblastoma cell lines were challenged with ZIKV MOI 1 for 24, 48, and 72 hour post infection, the percentage of infected cells were quantified by flow cytometry using 4G2 antibody.

Fig 2. Effect of MGMT overexpression and MGMTKO in ZIKV replication. (A) Relative expression level of AXL, MGMT and p53 in GMB cell lines. (B and C) MGMT overexpression and comparative ZIKV infection in GBM cell lines. (D and E) Expression of MGMT and ZIKV infection in MGMTKO MCF7 cell lines.

Fig 3. ZIKV infection and replication in MGMT-positive and p53 positive patient derived glioblastoma cell lines. Cell were challenged with ZIKV MOI 1 for 24, 48, and 72 hour post infection, the percentage of infected cells were quantified by flow cytometry using 4G2 antibody.

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