EGFR, but not EGFR expressed at low levels. The selectivity of mAb806 is thought to be due to its targeting a tumor-specific epitope of EGFR which is only accessible as the receptor transitions between an inactive tethered conformation to an active ligand-bound dimer. To address this question, we modeled several point mutations of the EGFR ectodomain (ECD) which naturally occur in GBM patients. Using molecular dynamics simulations we show that G63R and R108K expose the mAb806 epitope by way of their extreme flexibility at the domain I-II hinge region. In vitro analyses of these mutants reveal an increased ability to bind mAb806 in glioma cell lines. These point mutations and others were found to increase tumorigenicity in vivo. Examining downstream signaling pathways revealed similarities in signaling between the ECD deletion mutants EGFRvIII and EGFRvII while the induced point mutants R108K, G63R, A289Y and W1205X signal similarly to WT-EGFR. We go on to show that although treatment with a TKI before mAb806 increases binding to U87 parental and WT-EGFR expressing cells, and thus will likely prove to have increased toxicity in patients, forcing the kinase-inactive variant mAb806 would have different effects on mAb806 exposure based upon its mutational status. Thus, the ECD mutants may be used as a tool to increase the understanding of how EGFR structure affects function and ultimately oncogenicity.

CSIG-23. CYTOSOLIC RNA STRESS GRANULES: A PUTATIVE TRANSLATIONAL MECHANISM OF mTOR REGULATION IN GLIOBLASTOMA

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We performed RNA immunoprecipitation followed by microarray profiling of mRNAs pulled down by Stress Granule (SG) markers TIA1 and G3BP1 in normal and oxidative stress conditions to enrich for mRNA contained in SGs during stress in Glioblastoma cell lines. Interestingly, components of the mTOR complex, RAGG and LAMTOR were enriched in G3BP1 precipitates in stress conditions. We confirmed localization of RAGG mRNA to SGs utilizing single molecule RNA FISH in arsenite stressed Glioblastoma cell lines. We therefore hypothesized that perhaps RAGG mRNA are sheltered into SGs for rapid translation and stress response. We additionally found that protein levels of RAGG and LAMTOR increase at 25-35 minutes post-release from arsenite induced stress concomitant with a 50% decrease in SGs. We also observed similar increases in protein levels of other RAGG complex components (RAGA/B/C). This suggests translation of SG and mRNAs identified in our screen, which showed no increase in protein levels after stress release (TIA1, WAVE1/2, FOXX2, FOXX3). The increase in protein levels of the RAGulator complex components remained despite the addition of actinomycin D, suggesting that the increase protein levels are a result of translation of a stabilized cohort of mRNA and not de novo transcription. Interestingly, preliminary data utilizing a mCherry-LAMP construct suggests that mTOR colocalizes to the lysosome at 25-35 minutes post release from stress, a necessary step for mTOR activation. Utilizing Glioblastoma cells that we have engineered to display a delay in SG dissolution, we used a novel translational control mechanism of mTOR activation by SGs.

CSIG-24. REGULATION OF Fn14 EXPRESSION BY EGFR/III-STAT SIGNALING ENHANCES GLIOBLASTOMA CELL INVASION AND SURVIVAL

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Glioblastoma Multiforme (GBM) is the most common malignant brain tumor in adults. Most GBM patients succumb to the disease less than one-year post diagnosis due to the highly invasive nature of the tumor, which prevents complete surgical resection and gives rise to tumor recurrence. The invasive phenotype also confers radio- and chemoresistant properties to the tumor and cell lines; therefore, there is a need to develop new therapeutic strategies to control GBM invasion. Amplification of EGFR is observed in over 50% of GBM tumors, of which half concurrently overexpress the variant EGFRVIII, and expression of both receptors confers a worse prognosis. EGFR and EGFRVIII cooperate to promote tumor cell proliferation and invasion in part via the STAT-signaling pathway. Here we report that GBM cells expressing EGFRVIII show increased expression of a previously established mediator of glioma cell invasion and survival, fibroblast growth factor-inducible 14 (Fn14) at the mRNA and protein level. Treatment with STAT3 siRNA or Src inhibitors decreased Fn14 mRNA and protein expression. Finally, knockdown of Fn14 levels in the EGFRVIII-expressing glioma cells decreased both cell survival after temozolomide (TMZ) treatment and cell invasion, which suggests...