Phosphorylation state of Myristoylated Alkaline-Rich C-Kinase Substrate Effector Domain mimetics determines its cytotoxicity in glioblastoma and macrophage model

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ABSTRACT IMPACT: This study provides insight into how MED2 impacts the immune cells surrounding glioblastoma that help it to grow and spread; having a more complete understanding of how MED2 works will help us better develop therapies that may one day enter the clinic to improve patient outcomes in glioblastoma.

OBJECTIVES/GOALS: The purpose of this study was to determine whether the phosphorylation state of the MED2 peptide impacts its biological activity in GBM and macrophages. MED2 variants include the phosphorylatable wild-type (MED2), pseudo-phosphorylated (MED2-PP), non-phosphorylatable (MED2-NP) and control length (CTL2) peptides. METHODS/STUDY POPULATION: MED2, MED2-PP, MED2-NP, and CTL2 were screened against a panel of molecularly characterized glioblastoma patient derived xenografts and IL4/13 stimulated M2-like THP-1 macrophages. The luminescent cell viability assay, CellTiter-Glo, was used to determine viability.

RESULTS/ANTICIPATED RESULTS: The proneural lines XD456 and X1441 were highly sensitive to 5 μM MED2 and 5 μM MED2NP compared to 5 μM MED2PP (p<0.001). There was no statistically significant difference between untreated, 5 μM CTL2, and 5 μM MED2PP groups or between the MED2NP and MED2 treated groups. M2-like THP-1 macrophages were highly sensitive to 10 μM MED2NP compared to 10 μM CTL2 (p<0.01) and 10 μM MED2PP (p<0.01) No statistically significant difference was observed between untreated, 10 μM MED2, 10 μM MED2PP, and 10 μM CTL2 groups. DISCUSSION/SIGNIFICANCE OF FINDINGS: The phosphorylation state of MED2 determines its toxicity. When MED2 is phosphorylated, it is nontoxic to GBM or M2-like macrophages. The non-phosphorylatable version is toxic to both GBM and M2-like macrophages. The wild-type peptide is toxic to GBM but not M2-like macrophages, suggesting that MED2 may be phosphorylated in M2-like macrophages.

Antigen discovery in membranous glomerulopathy using laser capture microdissection and mass spectrometry*

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ABSTRACT IMPACT: Identifying the causative antigen in membranous glomerulopathy cohorts enables the development of serum assays to detect and monitor disease progression without the need for invasive kidney biopsies. OBJECTIVES/GOALS: Primary membranous glomerulopathy is caused by the formation of autoantibody immune complexes which deposit in the glomerulus and obstruct kidney function. Causative antigens remain to be identified in roughly 20% of cases. Our goal is to identify the antigen in these cohorts, so that non-invasive assays can be developed for disease monitoring. METHODS/STUDY POPULATION: Renal biopsy tissue from known antigen cases (PLA2R, THSD7A), and unknown cases were included in the analysis. Renal biopsy tissue from formalin fixed paraffin embedded tissue was cut at a thickness of 10 μm onto Leica PET-membrane frame slides. These slides were then stained with hematoxylin. The glomeruli were microdissected into microcentrifuge tubes using a Leica DM6000B microscope. The microdissected glomeruli were lysed in 2% SDS and 0.1M DTT at 99 degrees Celsius for 1 hour and processed by filter assisted sample preparation (FASP). Digested peptides were analyzed by liquid chromatography-mass spectrometry using an Orbitrap Fusion Lumos