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

Hasan Alrefai, BS, Nicholas J. Eustace, MD, PhD, Joshua C. Anderson, PhD, Patricia H. Hicks, BS, Peter H. King, MD and Christopher D. Willey, MD, PhD
The University of Alabama at Birmingham School of Medicine

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-NP, MED2-PP, 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.

20902

Elucidation of Cardioprotective Mechanisms via Human Models of Chemotherapy-Induced Cardiotoxicity

Nnamdi Uche, Qiang Dai, Shuping Lai and Ivor Benjamin
Medical College of Wisconsin

ABSTRACT IMPACT: This work should provide further insights to mechanisms of the negative consequences of chemotherapy drugs, specifically in the cardiovascular system. OBJECTIVES/GOALS: Cardiotoxicity remains a safety concern in the development or utilization of chemotherapeutics largely due to the gap in knowledge of the mechanisms of toxicity. The pathophysiology of this cardiotoxicity has not been fully elucidated but data from our lab as well as other recent studies hint toward implications of mitochondrial (mito) biogenesis. METHODS/STUDY POPULATION: Prophylactic use of the beta-blocker carvedilol as well as the ACE inhibitor enalapril have been shown to inhibit the development of anthracycline-induced toxicity, but the mechanism of this cardio-protection remains elusive. To explore this, human stem cell-derived cardiomyocytes and endothelial cells will be either treated with the anthracycline doxorubicin or pretreated with carvedilol or enalapril followed by doxorubicin treatment before cellular lysates are harvested. Western blotting and qPCR will be performed to determine the expression of mito biogenesis markers including Nrf1, TFAM and the master regulator of mito biogenesis, PGC-1α. RESULTS/ANTICIPATED RESULTS: We anticipate that doxorubicin treatment alone will result in decreased expression of the mito biogenesis markers Nrf1, TFAM and PGC-1α and that pretreatment with either carvedilol and/or enalapril prior to doxorubicin treatment will either prevent or reverse this. DISCUSSION/SIGNIFICANCE OF FINDINGS: Doxorubicin's role in causing mitochondrial dysfunction as well as suppression of biogenesis has already been established. Ideally, generation of new mitochondria would offset the occurrence of dysfunctional mitochondria. Confirming carvedilol/enalapril's involvement with mito biogenesis would provide a mechanism of cardio-protection.

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

Jacquelyn Fede[1], Stephen Kogut[1], Anthony Heyward[2], John F. Stevenson[1], Amy Nunn[3], Julie Plaut[3] and Judy A. Kimberly[3]
[1]University of Rhode Island, [2]Warren Alpert Medical School, Brown University and [3]Brown University

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

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