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POSTER ABSTRACTS

802.CHEMICAL BIOLOGY AND EXPERIMENTAL THERAPEUTICS

An Immunoproteasome Activator That Increases MHC Class I Antigen Presentation to Enhance Anti-Tumor Immunity

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Evasion of immune destruction is a hallmark of cancer that thwarts the efficacy of immunotherapy and reduces patient benefit. The development of immunotherapies has sparked a revolution in oncology by releasing the power of the human immune system, but the identity of novel, actionable antigens exclusively presented on cancer cells remains elusive. Proteasomes are multi-subunit proteolytic complexes that selectively degrade intracellular proteins. Proteasomes play a critical in controlling myriad cellular processes including metabolism, cell cycle progression, signal transduction, protein quality control and differentiation. Importantly, proteasomes are also the source of most antigenic peptides presented to the immune system on major histocompatibility complex (MHC)-class I molecules. In this process, intracellular and viral proteins are degraded in the cytosol to 8 and 9-amino acid peptides, which are then transported into the endoplasmic reticulum, where they associate with MHC-class I molecules and are presented to the cell surface. Immune cells contain a highly specialized, cytokine-inducible proteasome variant, known as the immunoproteasome, in which the three constitutive catalytic subunits are replaced by homologous catalytic subunits. Immunoproteasomes are specially adapted for a role in MHC class I antigen processing and presentation to CD8 $^{+}$ T-cells. Here, we performed a high-through screen (HTS) to detect novel molecular entities that activated immuno-proteasomal catalytic activity. We identified a curated panel of molecules which not only enhanced hydrolysis of the cell-permeable substrate LLVY-R110 but also enhanced the presentation of MHC class I antigens. E.G7-Ova is a mouse lymphoma cell line derived by electroporation of EL4 cells (C57BL/6, H-2b, T lymphoma) with chicken ovalbumin (*OVA*) cDNA. Proteasomes degrade OVA to generate the peptide SIINFEKL that is presented by murine MHC-class I H-2Kb molecules. E.G7-Ova cells were pretreated with compound A and co-cultured with the T-cell hybridoma B3Z that had been genetically-engineered to express a SIINFEKL-restricted t-cell receptor (TCR). Pretreatment of E.G7-Ova cells with compound A significantly increased tumor cell lysis by B3Z cells. The treatment of multiple myeloma (MM) cells with compound A also dramatically enhanced the expression of pan-MHC class I antigens. Treatment of MM cells with compound A increased the intracellular level of the proteasome activator PA28α/β, which is expressed by *PSME1/2*. Compound A treatment also enhanced hydrolysis of the fluorogenic peptide substrate Ac-ANW-AMC, which is selectively cleaved by the immunoproteasome β5-associated catalytic activity. Treatment of MM cells with compound A followed by mass spectrometry (MS) revealed that immunoproteasome activation modulated the relative presentation of individual antigenic peptides bound to MHC class I molecules. Pre-treatment of MM cells with compound A also significantly enhanced the anti-myeloma activity of human cytotoxic T-cells. Taken together, our results demonstrate that immunoproteasome activation has the potential to overcome immune escape and promote host immunity. In addition, our model systems now allows us to identify actionable tumor antigens on patient tumor cells as an indispensable tool for the development of cancer immunotherapies, e.g., T cell receptor (TCR)-transduced T-cells and patient-specific mRNA and peptide vaccines. Finally, immunoproteasome activators have the potential to contribute not only to cytotoxic T-cell mediated tumor lysis but also the treatment of intracellular infections and autoimmune and neurodegenerative proteinopathies characterized by the pathologic accumulation of toxic, proteinaceous aggregates.

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