innate immune response triggered upon oHSV treatment. This work suggests that β1 integrin inhibition may be utilized in the development of a novel oHSV therapeutic strategy.

658. Bortezomib Treatment Sensitizes Oncolytic Virus Treated Tumors to NK Cell Immunotherapy

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Background: Bortezomib, a proteasome inhibitor and oncolytic herpes simplex virus-1 (oHSV) are currently FDA-approved and continue to be evaluated in several human cancers. Various combinatorial treatment modalities are being investigated to enhance the efficacy of each treatment. In this study, bortezomib-mediated oHSV killing sensitization and anti-tumor immunity were evaluated. **Experimental Design:** The synergistic interaction between oHSV and bortezomib was calculated using Chou-Talalay analysis. Western blot, flow cytometry, and caspase 3/7 activity assays were used to evaluate the induction of necroptotic cell death, JNK activation, and apoptosis. Production of reactive oxygen species (ROS) was measured. Inhibitors/shRNA targeting ROS, JNK and RIP1 kinase (RIPK1) were utilized to investigate the mechanism of cell killing. Natural killer (NK) cells isolated from normal human blood and co-cultured with tumor cells at an Effect/Target ratio of 2:1. Q-PCR, ELISA, and FACS analysis were used to evaluate NK cell activation. Intracranial tumor xenografts were utilized to evaluate anti-tumor efficacy. **Results:** Combination treatment with bortezomib and oHSV induced necroptotic cell death with increased production of mitochondrial ROS and phosphorylation of JNK. Inhibitors/shRNA of RIPK1 and JNK rescued synergistic cell killing. Combination treatment also increased HMGB1 and IL-1α secretion and significantly enhanced NK cell activation and tumor cell killing. Moreover, combinatorial therapy enhanced NK cell therapy. **Conclusions:** This study provides a significant rationale for triple combination therapy of bortezomib, oHSV, and NK cells to achieve synergistic efficacy, leading to future clinical testing of oHSV with bortezomib in patients.

659. Oncolytic Adenovirus Loaded with Bioactive Modified Peptide as a Novel Approach to Treat Cancer

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Cancer is still a leading cause of death worldwide. Although many kinds of treatment have been developed during the past decades, there is still a lack of effective therapy for advanced cancer. Currently treatments such as surgery, chemotherapy and radiotherapy can help to improve patient prognosis and increase patient life expectancy. Therefore new treatment strategies against cancer are in high demand. Efficient anticancer agent and its targeted delivery into the tumor mass is a key prerequisite for the successful cancer therapy. Oncolytic virotherapy is emerging as a potential approach to treat cancer, using viruses, which are specifically engineered to selectively infect, replicate in and kill cancer cells without causing damage to normal cells. Their combination with chemotherapeutic agents have shown promising results due to the synergistic effect of viruses and drugs; therefore the combinatorial therapy is considered a beneficial approach for cancer treatment. Taken into account these considerations we optimized a strategy to conjugate peptides on the viral capsid, based on electrostatic interaction and used this strategy to deliver an active anti-tumor dipeptide. We used L-carnosine, a naturally occurring histidine dipeptide with anti-proliferative activity. A modified L-carnosine, positively charged was absorbed onto the viral capsid of an oncolytic adenovirus to generate a virus-carnosine complex. The complex showed enhanced anti tumor efficacy *in vitro* and *in vivo* and higher infectious titer compared to a naked oncolytic adenovirus in colorectal and lung cancer cells. The *in vivo* efficacy of the complex was analyzed in lung and colon cancer xenograft models, displaying a significant reduction in tumor growth and synergistic effect between virus and dipeptide. Moreover, we studied the molecular mechanisms underlying the effects of complex on tumor growth reduction. Complex can induce apoptosis in both cells lines, by using two different mechanisms, enhancing viral replication and affecting the expression of Hsp27. Our system could be used in further studies also for specific delivery of other active drugs.

660. Enhance Antitumor Effect by Combining Oncolytic Virus HF10 and Bevacizumab in the Treatment of Human Breast Cancer Xenograft

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Background: The high prevalence and poor prognosis of breast cancer provides a strong rationale for developing new treatment strategies. Oncolytic herpes simplex viruses has a promising prospect because of its selectivity, and the replicating and tumor killing ability. In our study, the antitumor effect by combining oncolytic virus HF10 and Bevacizumab in the treatment of human breast cancer xenograft is evaluated. Methods: The VEGFA gene transcription and protein expression were measured in candidate cell lines (MCF-7,T47D and MDA-MB-231) by RT-PCR, Western blot and ELISA. The MTT analysis was applied to evaluate the efficiency of the combination therapy in vitro. Viral replication was assessed by PCR and plaque assay. Animal models were formed by implanting MDA-MB-231 tumor in the flank site of female BALB/c nude mice. The HF10 group of advanced tumor model received two injections of 10^6 pfu/dose intratumorally on Day 1 and Day 14. The HF10 group of single tumor model received single injection of 10^6 pfu/dose intratumorally on Day 1. The Bevacizumab group received 5μg/g Bevacizumab intra-peritoneally twice a week for two weeks. The combination group received both intratumoral HF10 and intraperitoneal Bevacizumab at the same dose of single treatment groups. The tumor diameter was measured twice a week. On Day 3 and Day 36, the tumors were collected and observed respectively. Histopathological parameters were HIF1α, VEGFA, CD31 driven microvascular density, Caspase 3 and HSV-1 antigen. Results: MDA-MB-231 cells have the highest level of VEGFA expression, while T47D cells have the lowest level. The cytotoxic effect of HF10 is time- and dose- dependent in vitro. The combination therapy does not affect viral replication in vitro. The combination group has the smallest tumor volume comparing with other groups in both animal models ($P<0.05$). The combination therapy induces synergistic antitumor effect in both animal models. Viral distribution is significantly enhanced in the combination group compared to the HF10 group on both Day 3 and Day 36. Enhanced tumor hypoxia and the up-regulation of angiogenesis gene as well as enlarged population of apoptotic cells in the combination group are also demonstrated in the tumor sample on Day 3. Conclusions: Increased angiogenesis effect and limited viral distribution remain obstacles of oncolytic viral therapy. Anti-angiogenesis reagent is considered to be effective to achieve better antitumor effect of