621. Fusion Between Neutrophils (PMN) and Target Cells Mediate Cytotoxicity During Measles Virus (MV) Oncolysis - A Novel Mechanism

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We have previously shown that PMNs play a role in MV-mediated oncolysis (Grote et al) and that oncolytic vaccine strain of MV (as opposed to wild type) stimulates release of numerous cytokines with potential anti-tumor effects (Zhang et al). Recent data from our laboratory indicated that normal healthy PMNs were capable of specific killing of 35-40% of infected Jurkat target cells. On the basis that MV-induced cell-cell fusion amplifies IFN-alpha (α)/beta (β) production in infected cells, we sought to determine whether fusion between infected target cells and normal human PMNs specifically mediates cytotoxicity. First, we showed that PMN-mediated MV killing completely disappeared in the presence of FIP (fusion inhibitory peptide). Next, we examined the role of cell-cell fusion on PMN degranulation, reactive oxygen species (ROS) production and IFN production. We carried out all experiments with and without FIP. Jurkat cells were infected with MVNSe or mock-infected and incubated with PMNs isolated from healthy donors at 8:1 E:T ratio. At 24 hours, the cells were collected. PMN degranulation was determined by increase in cell surface expression of CD66b, CD63 and CD35 and ROS generation was determined by flow cytometry. IFN α and β production in the supernatant was quantified by ELISA. During co-culture of infected target cells and PMNs, IFN α (3000-5000pg/ml) and β (70-90pg/ml) were produced. No IFN α and β was produced by infected or uninfected Jurkats without PMNs present, confirming PMNs as the source. This was highly significantly abrogated in the presence of FIP (α - 200-300pg/ml; β - 0-3pg/ml). PMN degranulation and ROS generation followed the same pattern with abrogation in the presence of FIP, although the differences were not statistically significant. We confirmed that the α or β IFN was not directly responsible for cytotoxicity, since addition of the same quantity of exogenous IFN directly to Jurkat cells did not lead to cell death. Hence, we sought downstream cytotoxic effectors and evaluated TRAIL, which we know from our previous work, can be released from pre-formed granules in response to MV-infection of PMNs. The conditions that generated high levels of IFN also produced significant levels of soluble TRAIL (800-1500pg/ml), which was also abrogated in the presence of FIP (190-650pg/ml). Our data suggests infected target cells stimulate PMNs to display a cytotoxic effector phenotype, directly killing infected target cells. High levels of type I IFNs, are generated which are not in themselves responsible for the cytotoxicity. None of this occurs without target cell-neutrophil fusion as it is abrogated by FIP and does not occur in infected target cells alone or infected PMNs alone. This is a novel mechanism of stimulation of innate immunity by an oncolytic virus.

622. Oncolytic Adenoviruses Loaded With Active Drugs as a Novel Drug Delivery System for Cancer Therapy

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L-carnosine (β-Ala-His) is a naturally occurring histidine dipeptide, normally found in brain, kidney and in large amounts in muscle. L-carnosine has biological functions, including antioxidant activity, ability to chelate metal ions, as well as anti-inflammatory and anti-senescence properties. Recent studies have demonstrated that 50-100 mM of L-carnosine decreases cell proliferation in a colon cancer cell line HCT116, bearing a mutation in codon 13 of the RAS proto-oncogene. In addition, pre-treatment with L-carnosine decreases the intracellular concentration of Adenosine Triphosphate (ATP) and Reactive Oxygen Species (ROS) and inhibits the cell cycle progression in the G1 phase.The proto-oncogene KRAS is mutated in a wide array of human cancers and is important both in tumour progression and resistance to anticancer drugs. To overcome treatment limitations due to the high intracellular concentration required we have hypothesized that L-carnosine can be conjugated on the capsid of oncolytic viruses.Oncolytic viruses are viruses that are able to replicate specifically in and destroy tumor cells and this property is either inherent or genetically-engineered.The association of viruses with specific drugs, would increase the efficacy of the treatment of human neoplasia due to the synergistic action of virus and drug. First we have developed a strategy to conjugate peptides on viral capsid, based on electrostatic interaction. Then, using different cancer cell lines we found that oncolytic virus coated with L-carnosine with a tail of positively charged polylysine was able to enhance a positive anticancer synergistic effect. Finally, in order to investigate the molecular mechanisms underlying the effect of tumor reduction by oncolytic virus coated with modified L-carnosine, we have used three different approaches. First, we have examined, in samples with virus alone, or in combination with L-carnosine, the oncolytic replication by evaluating the E1A expression, second the apoptotic mechanism by expression of specific genes and at end the autophagy regulation via the amount of LC3-II. In conclusion, we have developed a model to use oncolytic adenovirus as a scaffold to deliver active drugs. Once validated the proposed model could be used as a novel drug delivery system for cancer therapy.

623. Immune Checkpoint Modulation Enhances Oncolytic Measles Virus Therapy

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We hypothesized that combining oncolytic Measles virus (MV) with immune checkpoint modulation using antibodies which block CTLA-4 and PD-1/PD-L1 signaling can provide synergistic anti-tumor effects.

Targeted immunomodulatory MV vectors encoding checkpoint modulating antibodies were cloned and characterized in vitro.

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