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Reovirus modulates autophagy during oncolysis of multiple myeloma

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Multiple myeloma (MM) is a clonal plasma cell malignancy that accounts for 10–15% of newly diagnosed hematological cancers. Although significant advances have been made in the treatment of MM the disease still remains incurable. The oncolytic potential of reovirus has previously been demonstrated by others and us and is currently in phase III clinical trials for solid tumors. In addition a phase I clinical trial has recently been initiated for MM. Despite the clinical activity, the mechanism(s) of cell death caused by reovirus in MM is yet not well elucidated. A comprehensive understanding of reovirus-mediated histology-specific cell death mechanisms is imperative if this therapeutic is to become a standard of care for patients. Previously we have shown that reovirus-mediated cell death of breast and prostate cancer is orchestrated via apoptosis. The present study demonstrates for the first time that in addition to inducing apoptosis reovirus also upregulates autophagy during oncolysis of MM.

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Multiple myeloma is a clonal neoplasm of plasma cells that accounts for approximately 10% of all hematological malignancies. Despite the advent of novel therapeutics such as thalidomide, revlimid and bortezomib as well as improvements made in the understanding of MM biology, the disease remains incurable. Therefore the need for novel, more effective and better tolerated therapeutics for MM is required.

Oncolytic viruses are a group of therapeutics that demonstrates an extensive range of anticancer activity in both solid and hematological malignancies. Of these, reovirus, a ubiquitous, nonenveloped, double-stranded RNA virus has proven minimal pathogenicity in humans while exhibiting extensive oncolytic potential against a myriad of cancers such as breast, prostate, brain tumors, renal carcinoma and hematological malignancies in vitro, in vivo, ex vivo and in clinical trials as has been demonstrated by us and others. Currently, reovirus is undergoing phase III clinical trial testing for head and neck tumors and phase I clinical trials have been initiated for multiple myeloma. The viral sensitivity of a wide array of tumor histologies is likely mediated via multiple permissive oncogenic signaling pathways that allow reovirus to target malignancies while sparing normal cells.

Autophagy is a catabolic process involved in homeostatic turnover of proteins and intracellular organelles that is not well understood in cancer tumorigenesis, promotion and response to anticancer therapeutics. Autophagy may in fact act as a tumor suppressor in the setting of early tumorigenesis, and as a cancer potentiator in established cancers including mediation of therapeutic resistance. Thus, many investigators have suggested strategies that combine both autophagic inhibition with cytotoxic chemotherapy or targeted therapies for potential synergy. In contrast, several lines of evidence suggest that ER stress leads to autophagy, and recent studies have highlighted a prominent role between autophagic cell death induced by AKT-MTOR signaling triggered by ER stress. A recent study has demonstrated that reovirus infection of MM leads to ER stress-induced apoptosis.

Therefore it is plausible to hypothesize that viral replication within MM, in addition to induction of apoptosis also promotes autophagic cell death initiated via a signaling mechanism of the ER stress pathway.

Recently we have shown the oncolytic potential of reovirus against several MM cell lines, ex vivo patient tumor, and demonstrated that reovirus could be used as a viable therapeutic for MM using a SCID/NOD murine model system. If reovirus therapy is to be optimized for patients it is imperative to understand sensitivity/resistant mechanisms of tumors. Previously we have shown that reovirus triggers apoptotic pathways in oncolyzing breast and prostate carcinomas. In the present study we demonstrate that in addition to inducing apoptosis, reovirus upregulates autophagy during oncolysis of MM, a novel finding that may link to synergy with other autophagy-directed strategies.

To examine the mechanisms of reovirus-induced cell death in MM, RPMI8226, NCI-H929 and U266 cells were treated with either no virus (NV), live virus (LV) or UV inactivated (dead) virus (DV) for 24, 48 or 72 h. Viable cell counts, DNA fragmentation and phosphatidylserine expression (annexin V binding), and active CASP3 were assessed via flow cytometry. Live reovirus treatment significantly enhances all apoptotic markers and dramatically reduces viable cell counts in MM cells in comparison to dead virus treatment. Caspase inhibition with Z-VAD-FMK-001 significantly reduces cell death in live reovirus treatments, but not in dead virus-treated MM cells, confirming reovirus-mediated apoptosis.

While apoptosis induction was prominent in reovirus-infected human myeloma cells, complete reovirus oncolysis could not be attributable to this process. We therefore hypothesized that autophagy may also be involved. RPMI 8226 cells were treated with NV, LV or DV for 0, 24 and 48 h. Cyto-ID Green Detection Reagent was utilized to identify vesicles colocalizing with LC3-II, a marker of autophagosomes and analyzed via flow cytometry. We observed that autophagy activity is similar in DV- and LV-treated RPMI 8226 cells at 0 h after virus infection. Autophagy induction is evident at 24 h in LV-treated cells demonstrating a 19% relative increase of median channel fluorescence (MCF) in comparison to NV treatment. This effect is more pronounced at 48 h leading to a 41% relative increase in median channel fluorescence. In contrast, the DV treatments showed only -4.0% and 5.3% relative changes in MCF in comparison to the uninfected controls. Autophagy was confirmed by treating RPMI 8226 cells with the autophagy inhibitor 3-methyladenine (3-MA), which downregulates this increase in autophagy caused by LV, resulting in a four-fold reduction by 48 h, suggesting that autophagy may contribute to cell death.

The novel finding that reovirus infection of MM leads to significant induction of autophagy prompts many critical questions that remain to be explored such as: (1) Is inhibition of autophagic pathways synergistic with reovirus for oncolysis? (2) What is the effect of MTOR inhibition in combination with reovirus on autophagic pathways? (3) Is this phenomenon unique to MM or similar with many different cancer histologies? (4) Are there activated cellular pathways such as RAS that are pivotal to this phenomenon? Interestingly, autophagy inhibitory drugs such as hydroxychoroquine are presently being tested in combination with cytotoxic chemotherapies and targeted agents. The rationale for these trials is that a majority of chemotherapeutics in vitro shows an increase in autophagy post-treatment, suggesting it acts as a prosurvival mechanism. Since autophagy is a dynamic process that could be histology specific and treatment specific it is intriguing to postulate that autophagy-inducing drugs such as verapamil or clonidine in conjunction with reovirus may in fact act synergistically in MM allowing better treatment options for patients. These avenues of research are worth exploration in the future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.