1126 MOLECULAR MECHANISMS OF DC ACTIVATION BY MELANOMA CELLS RESPONDING TO LTX-315

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Background Oncolytic peptides are emerging as attractive candidates for the development of novel anticancer regimens[1], reflecting broad cytolytic activities against a variety of malignant (but not normal) cells and a pronounced potential for immunostimulation.[2] LTX-315 is a synthetic nonameric cationic peptide derived from bovine lactotransferrin[3] that has been associated with a pronounced capacity to elicit tumor-targeting immune responses in various preclinical models of cancer.[2] Specifically, LTX-315 has been shown to (1) cause immunogenic cell death (ICD) [4] coupled to the release of immunostimulatory cytokines and damage-associated molecular patterns (DAMPs)[5,6], (2) deplete the tumor microenvironment (TME) of immunosuppressive cells such as $CD4^+CD25^+FOXP3^+$ regulatory T (T_{REG}) cells and myeloid-derived suppressor cells (MDSCs)[7], and (3) synergize with immunogenic chemotherapy[8] or immune checkpoint inhibitors (ICIs)[7] in the control of syngeneic mouse tumor models. However, the effects of tumor cells responding to LTX-315 on dendritic cells (DCs) remain to be precisely elucidated.

Methods Human A375 and mouse B16F10 cells were used as models of melanoma, *in vitro* (A375, B16F10) and *in vivo* (B16F10) upon subcutaneous injection in immunocompetent C57BL/6 mice. Flow cytometry, ELISA, RT-PCR and immunoblotting were used to assess DC maturation *in vitro* and *in vivo*, immunoblotting was employed to investigate the molecular mechanisms underlying DC activation, knockout and knockdown systems were harnessed to demonstrate mechanistic involvement. Therapeutic assays with B16F10 melanoma cells growing in immunocompetent vs *Myd88*$^{-/-}$ mice coupled to tumor rechallenge were employed to confirm activation of tumor-targeting immunity coupled to the establishment of immunological memory.

Results LTX-315 mediated the release of immunostimulatory DAMPs and nucleic acids (NAs) from melanoma cells that drove robust DC activation as monitored by surface immunophenotyping for CD80, CD83, CD86 and MHC class II and cytokine (TNF, IL-1β) secretion. This immunostimulatory effect mechanistically depended on TLR9 (detecting LTX-315-DNA complexes), TLR8 (detecting LTX315-RNA complexes) and TLR7 (which was activated by LTX-315 per se), as well as on the common TLR signal transducer MYD88. Accordingly, the *in vivo* anticancer activity of LTX-315 against B16F10 melanoma cells was significantly reduced in *Myd88*$^{-/-}$ mice, and *Myd88*$^{-/-}$ mice eradicating B16F10 tumors upon LTX-315 treatment remained susceptible to a rechallenge with B16F10 cells.

Conclusions In addition to induce immunogenic cell death, LTX-315 also activates DC via MYD88-dependent pathways that mediate optimal immunostimulatory effects coupled to the establishment of cancer-specific immunological memory in preclinical melanoma models.

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