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The Porcine Reproductive and Respiratory Syndrome Virus Genotype 2 (PRRSV-2)-derived Oncolytic Protein Reprograms Tumor-Associated Macrophages

Authors : Farrah Putri Salmanida, Mei-Li Wu, Rika Wahyuningtyas, Wen-Bin Chung, Hso-Chi Chaung, Ko-Tung Chang

Abstract : Within the field of immunotherapy, oncolytic virotherapy (OVT) employs dual approaches that directly eliminate tumor cells while preserving healthy ones and indirectly reprogram the tumor microenvironment (TME) to elicit antitumor responses. Within the TME, tumor associated macrophages (TAMs) manifest characteristics akin to those of anti-inflammatory M2 macrophages, thus earning the designation of M2-like TAMs. In prior research, two antigens denoted as A1 (g6Ld10T) and A3 (ORF6L5), derived from a complete sequence of ORF5 with partial sequence of ORF6 in Porcine Reproductive and Respiratory Syndrome Virus Genotype 2 (PRRSV-2), demonstrated the capacity to repolarize M2-type porcine alveolar macrophages (PAMs) into M1 phenotypes. In this study, we sought for utilizing OVT strategies by introducing A1 or A3 on TAMs to endow them with the anti-tumor traits of M1 macrophages while retaining their capacity to target cancer cells. Upon exposing human THP-1-derived M2 macrophages to a cross-species test with 2 µg/ml of either A1 or A3 for 24 hours, real time PCR revealed that A3, but not A1, treated cells exhibited upregulated gene expressions of M1 markers (CCR7, IL-1ß, CCL2, Cox2, CD80). These cells reacted to virus-derived antigen, as evidenced by increased expression of pattern-recognition receptors TLR3, TLR7, and TLR9, subsequently providing feedback in the form of type I interferon responses like IFNAR1, IFN-ß, IRF3, IRF7, OAS1, Mx1, and ISG15. Through an MTT assay, only after 15 µg/ml of A3 treatment could the cell viability decrease, with a predicted IC50 of 16.96 µg/ml. Interestingly, A3 caused dose-dependent toxicity to a rat C6 glial cancer cell line even at doses as low as 2.5 µg/ml and reached its IC50 at 9.419 µg/ml. Using Annexin V/7AAD staining and PCR test, we deduced that a significant proportion of C6 cells were undergoing the early apoptosis phase predominantly through the intrinsic apoptosis cascade involving Bcl-2 family proteins. Following this stage, we conducted a test on A3's repolarization ability, which revealed a significant rise in M1 gene expression markers, such as TNF, CD80, and IL-1ß, in M2-like TAMs generated in vitro from murine RAW264.7 macrophages grown with conditioned medium of 4T1 breast cancer cells. This was corroborated by the results of transcriptome analysis, which revealed that the primary subset among the top 10 to top 30 significantly upregulated differentially expressed genes (DEGs) dominantly consisted of M1 macrophages profiles, including Ccl3, Ccl4, Csf3, TNF, Bcl6b, Stc1, and Dusp2. Our findings unveiled the remarkable potential of the PRRSV-derived antigen A3 to repolarize macrophages while also being capable of selectively inducing apoptosis in cancerous cells. While further in vivo study is needed for A3, it holds promise as an adjuvant by its dual effects in cancer therapy modalities.

Keywords : cancer cell apoptosis, interferon responses, macrophage repolarization, recombinant protein
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