ImmunoTools *special* Award 2019

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Immunomodulatory role of regulated necrotic cancer cells

Background

Cells can die in different ways: as a result of natural processes, such as development or due to damage, infection or disease. As a textbook knowledge, the most well-known and the best characterized types are apoptosis and accidental necrosis, which show differences in both stimuli for induction and morphological and molecular changes. For a long time, necrosis has been considered to be unquestionably accidental and uncontrolled process. However, it is currently clear that necrotic cell death, is highly regulated process and covers several types of cell death. In fact, range of regulated cell death modalities is broad and includes over 12 sub-types of cell deaths [1].

In the last decade, it has become clear that anti-cancer therapy is more successful when it can also induce an immunogenic form of cancer cell death (ICD) [2]. In general, ICD is characterized by the emission of damage-associated molecular patterns (DAMPs, such as ATP, HMGB1, calreticulin, nuclear and mitochondria DNA) and/or cytokines/chemokines, leading to the induction of strong anti-tumour immune responses. ICD is rather an umbrella term covering several cell death modalities, including apoptosis and necroptosis.

Triggering necroptosis has become especially important in experimental cancer treatments as an alternative to triggering apoptosis, as one of the hallmarks of cancer is the blockade or evasion of apoptosis [3]. Recent reports from several laboratories, including ours, have stated that necroptotic cancerous cells can be perceived as a pro-inflammatory and immunogenic mode of cell death [4,5]. Although the induction of immunogenic necroptosis in cancer cells seems to be promising in

experimental mouse models in terms of activating anti-tumour immunity, it is important to stress that many cancers often develop necroptosis resistance as well [6]. Therefore, triggering immunogenic apoptosis or necroptosis would be not always the most optimal strategy. That is why it is of great importance to find ways to kill tumour cells by triggering other regulated necrotic cell death modalities.

Objective

The aim of my PhD project is to investigate immuno-modulatory role of cancer cells undergoing regulated necrotic cell death type with the immune system in the context of cancer and how cancer cells undergoing regulated necrosis modulate tumor microenvironment.

Specific objectives and Methods

I. *In vitro* analysis of regulated necrotic cancer cells immunogenicity:

We will investigate the maturation of murine bone marrow-derived dendritic cells (BMDCs) as a marker for immune cell activation. Briefly, murine BMDCs will be incubated with regulated necrotic cancer cells followed by flow cytometry-based analysis of phenotypic maturation markers like CD86, CD80, CD83, and HLA-DR/MHC-II (ImmunoTools). Then, we will probe the supernatants from viable/regulated necrotic cancer cells and after their coculture with BMDCs for following cytokines via ELISA (IL-6, TNFα, IL-10, IL-12p70, IL-1β).

II. I*n vivo* analysis of Infiltration of myeloid cells into the tumour bed:

For this MCA205 cells will be subcutaneously injected in C57BL/6 mice. Once the tumour size reaches 5x5 mm, the mice will receive the necroptotic inducer intra-tumoral, inducing necroptosis of the tumour cells *in vivo*. The infiltration of myeloid cells into the tumour will be monitored after 48 hours, when the tumours will be harvested, dissociated and stained for the different populations, before being subjected to flow cytometry analyses, as previously described (ImmunoTools). Macrophages will be identified as being positive for the markers CD11b, F4/80, CD169; neutrophils as $CD11b^{+}Ly6G^{+}Ly6C^{-}$ cells. The presence of $CD11b^{+}CD11c^{+}MHCII^{+}Ly6C^{high}$ dendritic cells will be verified, as it was shown to mediate the anti-tumour immune response in the context of ICD. Moreover, $CD11b^{+}F4/80^{+}CD169^{+}$ macrophages will be purified and evaluated for their M1 or M2

phenotype by extracting their RNA, converting it into cDNA, and performing real-time PCR of the following genes: Ccl17, Cx3cl1, Cxcl9, Cxcl10, Cxcl11, Ccl5, Nos2, Pgf, IL12a, Il1b, Tnf (M1-related genes) as well as Mrc1, Ccl2, Ccl7, Arg, Il10, Cxcl1, Cxcl2,Ccl3, Ccl4 (M2-related genes).

References

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