ImmunoTools *special* Award 2015

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Type I and III interferon signaling to enhance innate and adaptive anti-tumor immunity

The knowledge that the host's immune system can specifically recognize and eliminate malignant cells is the impetus for the fast-growing domain of cancer immunotherapy. Antigen-specific immunotherapy, such as dendritic cell (DC) vaccination, is found to be efficacious with only mild toxicity, but objective and durable clinical responses are confined to a minority of patients. Yet, further development of this form of treatment is highly desirable, especially considering that population ageing will lead to a strong increase in the number of new patients with limited tolerance towards more aggressive conventional cancer treatments. New strategies which improve effectiveness will therefore need to be pursued in order for antigen-specific cancer immunotherapy to reach its full potential in clinical practice.

Type I interferon (IFN) can bolster anti-tumor immunity, by restoring or increasing the function of DCs, T cells and natural killer (NK) cells, and can yield clinical responses in cancer patients. Moreover, type I IFN signaling on DCs was found to be essential in mice for tumor rejection by the innate and adaptive immune system. Also in our own experiments (manuscript under review), we found that IFN-α mRNA electroporation of monocyte-derived DCs significantly enhances the stimulation of tumor antigen-specific cytotoxic T cell as well as anti-tumor NK cell effector functions *in vitro*, through high levels of IFN-α secretion. Such local delivery of IFN-α by DCs to immune cells could boost anti-tumor immunity *in vivo*, while avoiding the side effects associated with systemic administration of IFN-α.

The production of type I IFN as well as the recently discovered type III IFN (IFN-λ) is controlled by IFN-regulatory factors (IRFs) following activation of pattern recognition receptors (e.g., Toll-like receptors (TLRs) and retinoic acid-inducible gene 1-like receptors (RLRs)) by certain pathogen-associated molecular patterns (PAMPs). In the current project we aim to exploit the TLR/RLR-IRF-IFN signaling pathway in DCs

to enhance anti-tumor immunity. Specifically, IRFs will be introduced into DCs followed by stimulation with specific PAMPs to trigger type I and III IFN production.

Human and murine DCs will be generated from peripheral blood $CD14^+$ monocytes and bone marrow mononuclear cells, respectively, according to a two-step protocol using IL-4/GM-CSF and TNF-α/PGE_2 ± sCD40L (ImmunoTools). These DCs will be genetically modified to express IRFs or stimulated with type I IFN (ImmunoTools) to induce IRFs. IRF-expressing DCs will then be exposed to different TLR/RLR agonists to trigger type I and III IFN production and used to stimulate NK cells and T cells *in vitro* and *in vivo*.

The effects of DCs on autologous NK cell responses against tumor cells will be evaluated *in vitro* using flow cytometer-based assaying and ELISA (ImmunoTools). NK cells stimulated with type I or III IFN (ImmunoTools) will serve as controls. Furthermore, DCs will be examined for their ability to expand tumor-reactive autologous T cells, identified using ELISA (ImmunoTools), through repeated stimulations in the presence of cytokines (ImmunoTools). Finally, IRF-expressing murine DCs ± TLR/RLR agonists will be investigated in a murine cancer model to determine their anti-tumor potential *in vivo*.

In summary, this project will generate knowledge about TLR/RLR-IRF-type I/III IFN signaling in anti-tumor immunity that could be applied to improve the effectiveness of cancer immunotherapy.