ImmunoTools *special* Award 2019

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Developing the IL-1 family member IL-36 as a novel therapeutic target for the treatment of colon cancer

Background

The importance of inflammation in cancer is now well established. In some cancers, the inflammatory conditions precede the development of malignancy, e.g., chronic bronchitis is a major risk factor for lung cancer. Alternatively, aberrant signalling due to oncogenic mutations in tumors can result in a chronic inflammatory state developing both proximal to, and within, the tumor. This chronic inflammation acts to inhibit the anti-tumorigenic immune response, normally mediated by cells such as M1 macrophages, NK cells and $CD8_+$ T cells. Tumor cells themselves can also directly induce an immunosuppressive microenvironment through recruitment and activation of specific immune cell further promoting tumorigenesis. Understanding the complexity of immunomodulation by tumors is important for the development of effective immunotherapies. Cytokines, such as IL-1 are mediators of the interactions between cells in the inflammatory tumor microenvironment. The IL-1 family now includes seven ligands with pro-inflammatory activity (IL-1a and IL-1b, IL-18, IL-33, IL-36a, IL-36b, IL-36g) as well as anti-inflammatory cytokines (IL-37, IL-38). Several members of this family, such as IL-1b and IL-18, have been extensively investigated in cancer with both pro- and anti-tumorigenic functions ascribed to these cytokines. In contrast, far less is currently understood concerning the role of more recently identified members of this family in cancer such as IL-33, IL-36 and IL-37, although such data that is available also indicates that these may have both pro- and anti-tumorigenic effects.

General Objective

The overarching aim of this project is to define the role of the novel IL-36 cytokines in tumorigenesis and to determine the potential therapeutic benefit of targeting this pathway in colon cancer.

Specific Objectives and Methodology

Objective 1. The functional effect of all the IL-36 cytokines in colon cancer cell lines will be investigated. Cells will be stimulated with the individual IL-36 cytokines and changes on cellular proliferation (BrDU incorporation), cell migration (transwell migration assay), and cell invasion (Matrigel Invasion assay) determined. Using qRT-PCR and ELISA (ImmunoTools), I will also examine whether there are differences between the three IL-36 cytokines in terms of their induction of pro- or anti-tumorigenic cytokines.

Objective 2. Elucidation of the role of IL-36 cytokines in colon cancer using a subcutaneous tumour model. We will generate stably transfected CT26 colon tumour cells that overexpress the individual cytokines. Both overexpressing and parental cells will be injected subcutaneously into mice and tumour growth monitored. At necropsy, tumours will be excised and tumour infiltrating lymphocyte populations will be assessed using flow cytometry. RNA will also be isolated from tumour tissue and the cytokine profile assessed by qRT-PCR.

Objective 3. Should we observe that signalling through the IL-36R exerts an anti-tumorigenic effect in vivo we will examine whether administration of IL-36 cytokines results in suppressed tumour growth, and as such represents a viable option for colon cancer therapy. Parental colon tumour cells will be injected subcutaneously as in *Objective 2*, and the relevant recombinant form of IL-36 administered intraperitoneally at regular intervals. Conversely, should we observe a pro-tumorigenic effect mediated by signalling through the IL36R we will administer the blocking antibody to the IL-36R to inhibit the biologic effects of IL-36R agonists

The role of Immuntools reagents in this study

Immune infiltration into the tumour micro environment will be investigated by flow cytometry and thus conjugated antibodies will be integral to achieving both identifying these populations and investigating their activity.

ELISA kits will prove very important in the characterisation of further cells lines in order to produce a more ubiquitous profile for colon cancer cells in vivo and in vitro. Examining protein production of cytokines using these kits will facilitate this for both murine cell lines and human cell lines.
The use of both human and recombinant cytokines will also play an important role in further investigating the expression profiles of IL-36 cytokines in further cell lines. Preliminary data thus far has indicated TLR ligands as potent activators of expression, however further cytokine stimulation is likely to augment the expression of these cytokines which will be important novel data.

ImmunoTools *special* AWARD for **Kevin Baker** includes 25 reagents:

human recombinant cytokines	rh IFN gamma, rh IGF-I,rh IL-1beta /IL-1F2, rh IL-6 rh IL-8/CXCL8, rh IL-10, rh IL-36A/IL1F6, rh MCP-1 / CCL2, rh MIP-3a / CCL20, rh TNFα, rh VEGF-A/VEGF-165, rh CTLA-4 / CD152
human ELISA-set	IFNgamma, IL-1beta
mouse ELISA-set	TNFalpha
mouse recombinant cytokines	rm GRO-a / CXCL1

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