ImmunoTools *special* Award 2019

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Tryptophan 2,3-dioxygenase inhibition: a possible target in cancer immunotherapy

Background

It is known that tryptophan (trp) degradation is a mechanism employed by a broad range of tumours to suppress immune response. Tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) catalyze the first and rate limiting step of trp oxidation yielding kynurenine (KYN). IDO expression is upregulated in melanoma contributing to immunologic evasion (1). T lymphocytes sense low levels of TRP, block their proliferation and differentiate into Treg, leading to immunosuppressive microenvironment (2). IDO1 is reported to make antigen presenting cells tolerogenic to promote tumorigenesis and help build immune checkpoints in cancer [3]. Some IDO inhibitors have been/are currently being tested in humans in phase 1 and 2 trials, and their benefit have not completely demonstrated, suggesting a role for TDO (4). Recent studies propose an alternative route of trp degradation in tumours via TDO. Pharmacologic inhibition with the selective TDO inhibitor 680C91 increased cellular sensitivity to anoikis, and reduced TNBC proliferation, migration, and invasion in a breast cancer cell line (5). TDO inhibition may represent an additional compelling target for cancer immunotherapy. This project aims to extend the knowledge in TDO role on immune function and tumor biology studying the expression and function of TDO on human DCs and SKMel28 melanoma cell line, that we have recently demonstrated express TDO (manuscript in preparation). TDO has been found in in human solid tumors and in some cancer cell lines, however its inhibition on latter function, in vitro, is still under investigation.

Objectives

1. Characterize TDO function in SKMel28 melanoma cell line, since no data are reported yet: cell proliferation and invasion in the presence of its selective inhibitor 680C91 will be tested;

2. Since TDO promotor possesses glucocorticoids responsive elements (GR) sequences, we aim to verify whether TDO mRNA could be modulated by dexamethasone.

3. Assess TDO mRNA and protein expression in response to cytokines which are elevated in melanoma patients, such as CCL3, CCL4 and IFNgamma;

4. Characterize TDO expression and its modulation by dexamethasone in human dendritic cells (DCs).

5. Evaluate DC maturation and function in the presence of TDO inhibitor, 680C91.

Methods

AIM 1: SKmel 28 will be stimulated with FGF-2 or PDGF-bb in the presence or absence of 680C91 in order to assess cell proliferation (cell duplication), cell apoptosis and cell cicle by means of flow cytometry (**ImmunoTools**).

AIM 2: SKmel 28 will be stimulated with dexamethasone and mRNA TDO will be assessed by means of real-time PCR

AIM 3: SKMel 28 will be stimulated with IFNgamma or CCL3, CCL4 then TDO expression will be assessed by real-time PCR and Western blot.

AIM 4: DC obtained from CD14+ precursors will be stimulated with dexamethasone and TDO mRNA will be measured; DC maturation will be assessed by means of flow cytometry for maturation markers (CD80, CD86, CD83) (**ImmunoTools**).

AIM 5: DC maturation in the presence of TDO inhibitor will be assessed by means of flow cytometry (**ImmunoTools**) and ELISA for IL12 amd IL-10 production (**ImmunoTools**), able to induce TH1 or Treg generation.

1. Beatty et al., Clin Cancer Res. 2015 Feb 15;21(4):687-92.
2. Opitz CA et al. Nature. 2011;478:197-203.
3. Mellor et al., Front Immunol. 2017; 8: 1360.
4. Platten et al, Front Immunol. 2015 Jan 12;5:673.
5. D'amato et al., Cancer Res. 2015 Nov 1;75(21):4651-64.

ImmunoTools *special* AWARD for **Astrid Parenti**, includes 25 reagents

FITC - conjugated anti- human	CD11c, HLA-DR, Annexin V
PE - conjugated anti- human	CD4, CD80
PerCP- conjugated anti- human	CD3
APC- conjugated anti- human	CD8, CD86, Annexin V
recombinant human cytokines	hFGF-b / FGF-2, rh GM-CSF, rh IFNgamma, rh IL-4, rh IL-6, rh MIP-1a / CCL3, rh MIP-1b / CCL4, rh PDGF-BB, rh TNFα
human ELISA-sets	human IL-10, human IL-12p40 total

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