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Short Communication Open Access

IL4I1: An Emerging Target to Reinvigorate Antitumor Immune Responses

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Short Communication

One of the known cancer-promoting mechanisms dependent on the microenvironment consists of the expression of amino-acid catabolizing enzymes. The enzyme Interleukin-4 Induced gene 1 (IL4I1) is a secreted L-phenylalanine oxidase physiologically produced by antigen presenting cells of the myeloid and B cell lineage and in T helper type 17 cells. *IL4I1* inhibits T cell proliferation and cytokine production, and facilitates naïve $CD4^+$ T cells differentiation into regulatory T cells *in vitro*, in part *via* its capacity to produce H_2O_2 and deplete phenylalanine from the T cell microenvironment. In humans, *IL4I1* is expressed in tumor-associated macrophages of most cancers and in tumor B cells of lymphoma originating from the germinal center. As shown in a mouse model of melanoma, its local production may facilitate human tumor growth by inhibiting the anti-tumor $CD8^+$ T cell response. All these data, and others that are developed in this short review, point to *IL4I1* as a new drugable target in the field of immunomodulation in cancer.

The research of the last decades has highlighted the dual role of the immune system in cancer development. Whereas the induction of a specific antitumor immune response can play an active role in cancer cell elimination [1], the selective pressure it exerts leads to the development of immunosuppressive mechanisms that facilitate subsequent tumor cell escape from the immune control. This last immunosubversive ability, which has been included as a cancer hallmark in 2011, offers new potential targets for therapeutic intervention in patients [2,3].

Different types of leukocytes infiltrate tumors and can affect local immune responses, but tumour-associated macrophages (TAMs) usually predominate and their density or transcriptional signature has been associated with poor prognosis and reduced survival in a large number of cancers [4,5]. One of the major TAM cancer promoting mechanism consists of the expression of amino-acid catabolizing enzymes [6]. The best-characterized enzymes belonging to this group, i.e. indoleamine 2,3 dioxygenase (IDO), arginase 1 (Arg 1) and inducible nitric oxid synthase (iNOS) participate in the inhibition of the adaptive immune response by decreasing essential or semi-essential amino acids and producing different toxic metabolites in the lymphocyte environment, thus affecting their proliferation and function. Expression of these enzymes in the tumor bed and/or in lymphoid organs has a negative impact on survival. This can be reversed by the use of specific inhibitors in pre-clinical models, justifying the current phase I/II clinical trials of IDO inhibitors.

Interleukin-4 induced gene I (*IL4I1*) was initially described as an early-induced gene by IL4 treatment of B splenocytes [7] with high homology to L-amino acid oxidases. This protein corresponds to isoform 1, whose expression is restricted to lymphoid tissues [8]. In 2005, a second isoform, was described in rare cells of nervous tissue (e.g. Purkinje cells) and of the testis (Sertoli cells) [9]. Since then, three other isoforms have been referenced in databases. All these isoforms differ in the first exons encoding their respective signal peptides that should be cleaved at homologous positions in the proteins. Consequently, the five proteins should have identical sequences once processed.

Figure 1: L-aminoacid oxidation catalyzed by *IL4I1*. *IL4I1* degrades L-phenylalanine (Phe) to produce phenylpyruvate, hydrogen peroxide (H_2O_2) and ammonia (NH_3). An alternative substrate of *IL4I1* is arginin (not depicted).

Following the discovery of *IL4I1* expression in primary mediastinal B cell lymphoma [10], we have shown that the isoform 1 of *IL4I1* is a glycosylated and secreted protein endowed with an L-amino acid oxidase activity [11]. Both mouse and human *IL4I1* degrade phenylalanine to produce the corresponding α-keto acid (phenylpyruvate), H_2O_2 and NH_3 (Figure 1). We also recently showed that the human form could catabolize arginine and identified specific SNPs and mutations in the *IL4I1* coding sequence, which determine hyperactive or hypomorphic *IL4I1* molecules [12]. These SNP may impact the immune response of affected individuals, since the *IL4I1* enzyme exerts immunosuppressive functions. Indeed, our first work indicated that the *IL4I1* enzymatic activity, including the H_2O_2 produced, inhibits the proliferation of T cells, particularly effector/memory ($CD45RO^+$) T cells, and decreases the production of inflammatory chemokines and Th1 cytokines (IFNγ and IL-2) (Figure 2) [11,13]. In humans, *IL4I1* is expressed by mononuclear phagocytes, i.e. dendritic cells, monocytes and macrophages, after stimulation of the STAT1 and/or NF-κB pathways by IFNγ and Toll-like receptor ligands (Figure 2). *In vitro*, a much lower level of *IL4I1* activity is also measured in peripheral blood B cells stimulated via the STAT6 and/or NK-κB pathways (e. g. with a cocktail of IL-4 and CD40 ligand which mimics stimuli received by germinal center B cells), but not in unstimulated peripheral blood T cells [13]. *In vivo*, *IL4I1* expression is detected in macrophages and/or dendritic cells from human lymphoid tissues and inflammatory lesions including tumors.

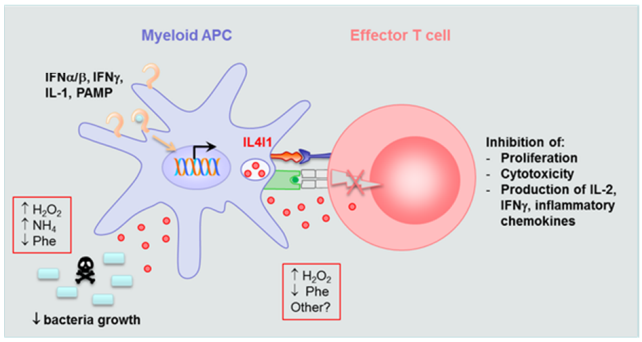

Figure 2: *IL4I1* effects on effector T cell activation: The production of *IL4I1* is induced in myeloid antigen-presenting cells (APC, i.e. dendritic cells, monocytes and macrophages) by proinflammatory mediators, such as pathogen-associated molecular patterns (PAMP), interleukin 1 (IL-1), type I interferons (IFNα/β) and IFNγ, which activate the STAT1 and/or NFκB pathways after binding to toll-like receptors, the IL-1 receptor, the IFNα/β receptor and the IFNγ receptor, respectively. *IL4I1* enzymatic activity depletes the local milieu in Phe. Phe depletion and increased concentration of H_2O_2 combined with medium basification by NH_3 can be toxic for bacteria, thus participating to an anti-infectious response. Phe depletion and H_2O_2 also affect effector CD4+ and CD8+ T lymphocyte activation, leading to diminished proliferation and effector functions. In the context of a tumor, the Th1 response may lead to *IL4I1* induction in TAM, which can then inhibit the antitumor T cell response.

Through an analysis of a series of 315 tumors, representing 30 different histological types, we have shown that *IL4I1* is strongly expressed by TAM and, in some cases, by neoplastic cells (mesothelioma and several B-cell lymphoma subtypes including follicular lymphoma, Hodgkin lymphoma, proliferation centers of chronic lymphoid leukemia) [14]. In the TAM, *IL4I1* expression may be induced by the local production of IFNγ by antitumor T cells. Indeed, we did not observe the expression of *IL4I1* in macrophages stimulated *in vitro* by T helper type2 (Th2) cytokines such as IL-4 and IL-13 and in macrophages present in Th2 inflammatory lesions, such as schistosomiasis-associated granulomas [13]. This is in contrast to what was observed in mouse macrophages, where *IL4I1* has been detected in M2 macrophages *in vitro* [15] and at the onset of inflammation resolution in mouse central nervous system lesions [16]. Differences between mice and humans which have also been observed in the expression of the arginine catabolizing enzyme, Arg1 [17], may explain these discrepancies. Interestingly, data obtained from a transcriptomic comparison, in mouse and human, of the three different skin migratory DC subpopulations with lymphoid organ resident DC (i. e. mouse lymph node $CD8\alpha^+$ DC and their proposed human homologues, blood $BDCA3^+$ DC) indicated that *IL4I1* was one of the genes most differentially expressed. In this work, migratory DC (which highly expressed *IL4I1*) were suggested to dampen the immune response induced by resident DC (which poorly expressed *IL4I1*) [18].

IL4I1 is expressed by $CD4^+$ T cells in some circumstances. While it is not detected in Th1 and Th2 subsets [7], the Annunziato's group has shown that the *IL4I1* mRNA is induced in Th17 cells by the RORγT master gene [19]. In this cell subset, *IL4I1* participates in controlling IL-2 production and cell proliferation in part by maintaining high levels of the transcription factor Tob1, which prevents TCR-dependent cell-cycle progression (Figure 3) [20]. *IL4I1* is also expressed by $FoxP_3^+$ Aiolos+ regulatory T cells and may modulate the pathogenicity of this cell population during inflammation-induced Th17 trans-differentiation (Figure 3) [21]. We recently confirmed that Th17 cells express active *IL4I1* by detecting its enzymatic activity in *ex-vivo* sorted human Th17 populations (unpublished data) and in *in vitro* generated Th17 cells where its production appeared to be induced in an autocrine manner (Figure 3) [22]. Expression of *IL4I1* in Th17 cells infiltrating some human cancers may contribute to their immunosuppressive and tumor-promoting properties [23]. Moreover, we have shown that *IL4I1* biases naive $CD4^+$ T cell differentiation towards that of $FoxP_3^+$ regulatory T cells *in vitro* (Figure 3). Induction of regulatory T cells was associated with the inhibition of signaling *via* the mammalian target of rapamycin complex 1 (mTORC1) pathway, but did not impact mTORC2. Thus, local *IL4I1* production may have consequences on the balance of effector *versus* suppressive T cells in the tumor microenvironment [13,22]. All these findings indicate that *IL4I1* represents a key immunoregulatory molecule of different T-cell functions [24]. In addition, *IL4I1* may play a role in the maturation of the T-dependent humoral response, since it is expressed by light zone germinal center B cells [25]. In these cells, it has been shown that *IL4I1* is a target of mutations produced by the activation-induced cytidine deaminase [26]. This might have consequences on *IL4I1* enzymatic activity and immunoregulatory properties. The expression of *IL4I1* in innate lymphocyte subtypes has not yet been explored.

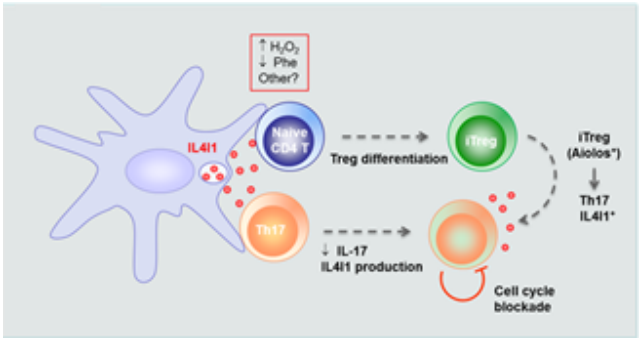

Figure 3: *IL4I1* effects on the balance between effector and regulatory CD4+ T cells: *IL4I1* production by dendritic cells during the priming of naïve CD4+ T cells favors regulatory T cell (Treg) differentiation. In the presence of Th17 inducing cytokines, *IL4I1* biaises Th17 differentiation towards cells that secrete less IL-17 and aquire the capacity to produce *IL4I1*. *IL4I1* production by Th17 blocks cell cycle progression, thus autolimiting the proliferation of this cell subset. Finally, circulating induced Treg cells (Ailos+ iTreg) can express *IL4I1* when converting into Th17 cells under inflammatory conditions. Local Phe depletion, which inhibits mTORC1 signaling, and H2O2 production participate in amplifying Treg cell induction from naïve T cells, but other yet undefined mechanisms are suspected. *IL4I1*-mediated immune escape of tumors may thus rely both on effector T cell inhibition and Treg cell induction.

To study the effect of *IL4I1* production in the context of a tumor, we have developed a preclinical murine melanoma model based on B16 cells transfected to express *IL4I1*. Following vaccination against a tumor antigen, we observed that *IL4I1* expressing-tumors developed

more frequently when compared to control tumors. This tumor escape was associated with the inhibition of proliferation and IFNγ production of antitumor cytotoxic $CD8^+$ T lymphocytes [27]. The minimal *IL4I1* activities leading to this phenomenon were close to those detected in primary human melanoma, a tumor where the *IL4I1* activity is supported exclusively by TAM. In line with these results, we recently observed that tumor growth was more easily controlled in the absence of the enzyme in *IL4I1* KO mice [28] and unpublished data. Moreover, in the absence of *IL4I1*, the growth of primary tumors and metastases was delayed in a model of spontaneous melanoma induced by the Ret oncogene. This delay was accompanied by modifications of the primary tumor infiltrate, with an increase in Th1 cells and cytotoxic T cells and a diminution of polymorphonuclear myeloid-derived suppressor cells [28]. Finally, in a HER2/neu positive mouse mammary carcinoma model, expression of *Il4i1* in the microenvironment was associated with tumor recurrence [29,30]. In accordance with these preclinical data, Finak et al. identified *IL4I1* as one of the 103 genes associated with pejorative prognosis, in a study of the genes expressed by the micro-dissected tumor stroma of human breast cancers. No other immunosuppressive enzyme-coding transcript was predictive of clinical evolution [31]. *IL4I1* gene expression is also significantly up-regulated in laser-microbeam dissected triple negative breast cancer cells compared to normal ductal cells [32] and in fibroblasts present in precancerous mammary lesions [33], suggesting that *IL4I1* may be expressed by breast cancer cells and tumor-associated fibroblasts in addition to TAM.

We provided evidence that the *in vitro* immunosuppressive effect of *IL4I1* is mediated through the production of H_2O_2 but, as for other amino-acid degrading enzymes, deprivation of an essential amino acid can also be involved [11,22]. *IL4I1* presents some ancestral anti-bacterial function, as other enzymes of the L-amino acid oxidase family, which are involved in anti-infectious defense in primitive species such as mollusks or reptiles. We have observed that this anti-bacterial effect is principally mediated by the toxicity of hydrogen peroxide, which can be amplified by medium basification due to ammonia production [34]. However, we did not observe such an effect of ammonia on T lymphocyte activation (unpublished data). We currently favor the hypothesis that different mechanisms cooperate to limit effector T lymphocyte functions and facilitate the expansion and differentiation of Tregs in tumors.

Stimulation of the antitumor immune response is gaining huge interest in clinical settings as spectacular successes have been obtained with the use of immune checkpoint inhibitors. However, not all patients experience long-lasting remissions and some cancer types respond poorly to this class of therapeutics. New immune targets are currently under clinical evaluation and others, such as *IL4I1*, may deserve careful exploration. Indeed, the data presented above argue that *IL4I1* could play an important role in dampening the anti-cancer immune response and understanding and blocking its activity may therefore be a promising strategy to improve current chemotherapy and immunotherapy approaches in cancer treatment. Moreover, the absences of overt autoimmune manifestations in *IL4I1* knock-out mice (unpublished data) suggests that such a strategy would not lead to major immune adverse effects in patients.

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