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Dysregulation of anti-tumor immunity by the matrix metalloproteinase-2

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The matrix metalloproteinase-2 (MMP-2), over-expressed in most cancers, induces T_H2 polarization by conditioning dendritic cells to over-express OX40L and down-regulate IL-12p70 through the degradation of the type-I IFN receptor IFNAR1. Elucidating mechanisms underlying detrimental tumor-associated type-2 responses represent a crucial step in designing effective immune therapies to treat cancer patients.

Matrix metalloproteinase-2 (MMP-2), a proteolytic enzyme that degrades extracellular matrix, is a self antigen overexpressed in several cancers including melanoma. Its level of expression and activation is associated with increased dissemination and poor survival/prognosis.[1,2] The protumoral functions of MMP-2 make it an appealing target for cancer therapy. In the context of immunotherapy, targeting such an antigen would limit tumor escape due to antigen loss variant selection. Interestingly, we previously established the existence of MMP-2-specific cytotoxic $CD8^+$ T cells in the tumor of melanoma patients.[3]

As CD4 help appears crucial for generating effective anti-tumor immunity, we investigated the existence of MMP-2-specific $CD4^+$ T cells. We detected such cells in healthy donors and more importantly at a much higher frequency in over 40% of melanoma patients tested. We cloned $CD4^+$ T cells specific for 11 novel MMP-2-derived epitopes. Strikingly, these cells displayed an inflammatory T_H2 phenotype, i.e., mainly secreting TNFα, IL-4 and IL-13 and expressing GATA3. Whereas IFNγ-secreting T_H1 cells appear to exhibit effective anti-tumor properties, T_H2 cells are often described as detrimental in this function.[4-6] Despite a lack of statistical significance, we also observed a trend toward a poorer clinical outcome/survival for patients with detectable MMP-2-specific T_H2 cells, suggesting the significance of these cells and/or MMP-2 itself in melanoma progression.

We investigated the mechanism underlying the observed T_H2 skewing and found that MMP-2-conditioned dendritic cells (DCs) preferentially prime inflammatory T_H2 cells. Of note, MMP-2 enzyme in its active conformation was the strongest inducer of T_H2 responses. While, according to the literature, T_H1 differentiation reliably depends on IL-12, the generation of T_H2 cells is not fully understood and/or may differ from one model to another. IL-4, through GATA3 induction, is described as critical for T_H2 differentiation in most studies. However, IL-4 did not seem to be involved in our system. A default mechanism could also explain T_H2 differentiation, where the lack of T_H1-polarizing signal, namely IL-12, would be sufficient and/or necessary.[7] Strikingly, we found that DCs exposed to active MMP-2 lost their ability to produce IL-12p70. Moreover, we demonstrated that this lack of IL-12p70 actually played a major role in the MMP-2-dependent T_H2 polarization. Indeed, DCs exposed to active MMP-2 lost their ability to prime T_H2 cells when rhIL-12p70 was supplemented in the culture. Rather, $CD4^+$ T cells differentiated into T_H1-like cells. We characterized the mechanism behind MMP-2-dependent inhibition of IL-12p70: by degrading the type I IFN receptor (IFNAR1) on DCs, and subsequently preventing STAT1 phosphorylation, MMP-2 inhibits IL-12p35 subunit transcription (**Fig. 1**).

Furthermore, we established that MMP-2 induced DCs to overexpress OX40L, which was also involved in the observed T_H2 skewing, as shown by performing the same priming experiments in the presence of a blocking antibody for OX40L: naïve $CD4^+$ T cells primed against MMP-2 when OX40L is being blocked, differentiated into IFNγ-secreting T_H1-like cells (**Fig. 1**). Despite evidence suggesting that OX40 signaling in $CD4^+$ T cells can directly induce type-2 lineage commitment by inducing NFATc1, which triggers IL-4 production and subsequent GATA3 expression,[8] the role of OX40L in T_H2 differentiation is not yet clearly understood. MMP-2-induced type-2 differentiation likely works differently as we showed that IL-4 was not a major determinant in this model. Thymic stromal lymphopoietin (TSLP) can also promote T_H2 differentiation through OX40L-expressing DCs. Interestingly, MMP-2 did not induce DCs to produce TSLP, implying that MMP-2-induced expression of OX40L does not depend on TSLP. TSLP-dependent T_H2 differentiation is believed to involve basophils in several models,[9,10] but we could not detect any basophil activation by MMP-2. We are now exploring how MMP-2 induces OX40L expression and are trying to identify the responsible receptor(s). Surface molecules such as αvβ3, CD91 or MT1-MMP are known

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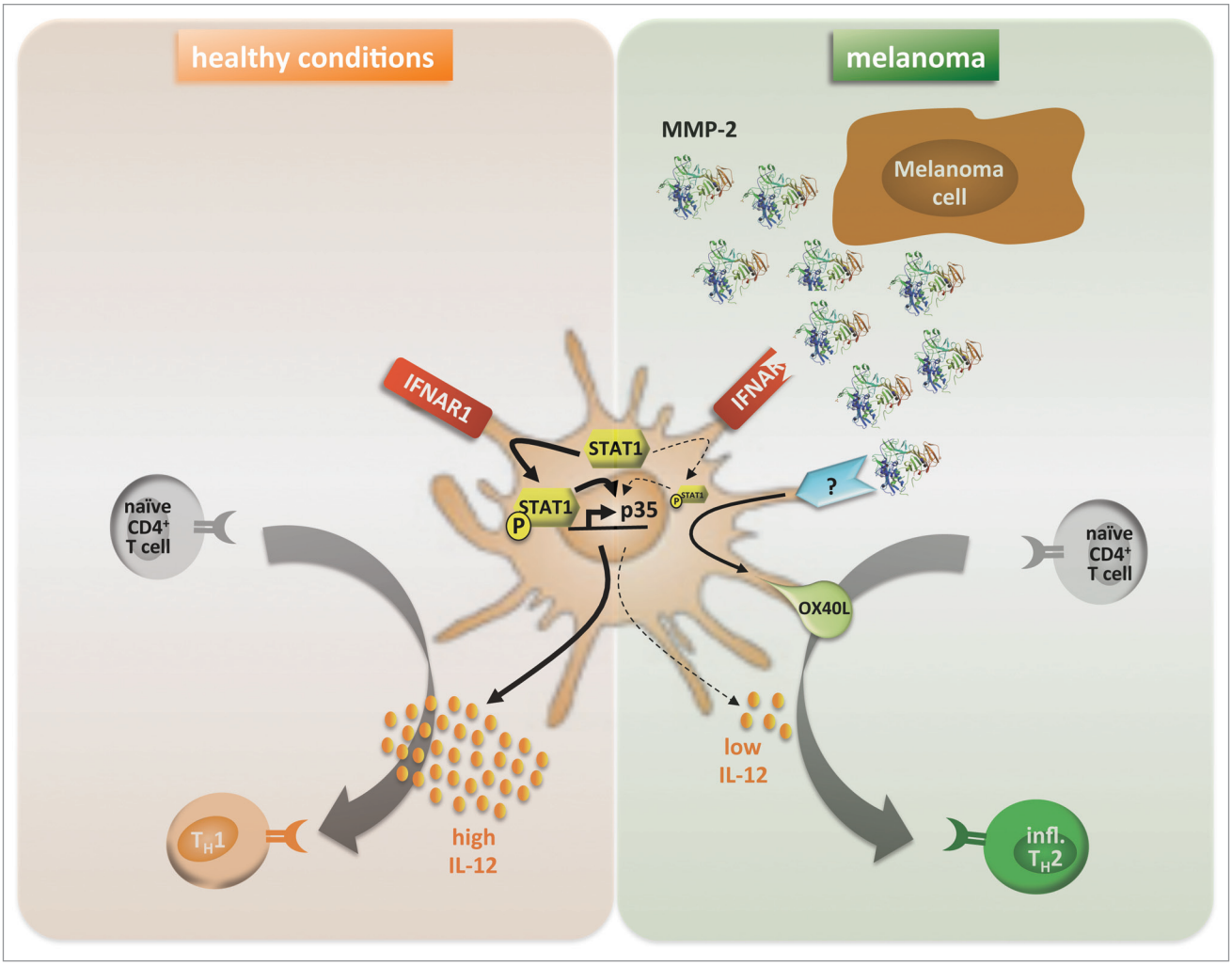

Figure 1. MMP-2-dependent mechanism licensing DCs to prime inflammatory T_H2 cells. The left part (orange) represents «healthy conditions»: activated DCs produce IL-12p35 through IFNAR1 and STAT1 signaling. DCs secrete high levels of IL-12p70 necessary for priming naïve CD4⁺ T cells into T_H1 cells. In the context of melanoma (right part, green), tumor cells produce and activate MMP-2. Active MMP-2 degrades IFNAR1 on DCs, leading to low levels of STAT1 phosphorylation preventing transcription of IL-12p35. Furthermore, MMP-2 in both active and inactive conformations induces OX40L overexpression on the surface of DCs through triggering of a receptor yet to be determined. Both lack of IL-12p70 and OX40L overexpression by MMP-2-exposed DCs are responsible for priming naïve CD4⁺ T cells into inflammatory T_H2 cells.

to bind MMP-2. However, we excluded any role for these receptors in OX40L induction.

Together, our priming results suggest that both the lack of IL-12 and OX40L overexpression play distinct and additive roles in our model. Moreover, OX40L blockade had an effect even in the presence of IL-12p70, indicating that the absence of IL-12 is not strictly required for T_H2 polarization in this model.

While OX40L expression is induced after exposure to MMP-2 in both active and inactive conformations, IL-12p70 blockade only occurs in the presence of active MMP-2. Aggressive tumors display high levels of activated MMP-2 and therefore should license in situ DCs to express OX40L in the absence of IL-12, creating, an ideal environment to generate T_H2 cells, according to our model. Whether T_H2 polarization in tumors is only a consequence of the environment or actually plays a part in tumor aggressiveness by restricting immune control still needs to be determined.

An additional major finding of our study is that MMP-2 acts as a type-2 conditioner for CD4⁺ T cells specific for other tumor antigens, as we showed for Melan-A/MART-1 and NY-ESO-1. One could imagine that MMP-2-exposed DCs would turn naïve CD4⁺ T cells of any specificity into type-2 cells. This would imply the generation/accumulation of a local type-2 tumor micro-environment, potentially reinforcing itself through second-hand IL-4 secreted by resident T cells.

Unraveling the mechanisms underlying tumor-associated T_H2 polarization, including the one we characterized in this study, opens the way to developing new therapeutic strategies for cancer patients able to induce effective type-1 responses and limit tumor cells escaping the immune system.

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