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Melanoma immunoediting by NK cells

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The immune system can control the early steps of tumor growth, but it may also induce phenotypic/functional changes of malignant cells during tumor progression, favoring immunoescape mechanisms. In a recent study, we revealed how natural killer (NK) cells can participate in such an immunoediting process, by rendering melanoma cells resistant to NK-mediated killing.

One of the major obstacles for defining efficient immunotherapeutic approaches for the treatment of solid tumors is the capability of the tumor microenvironment of inhibiting the host immune response both at local and systemic levels.[1] Even during the early steps of tumorigenesis, malignant cells can produce factors that are capable of influencing multiple components of the developing tumor stroma. Thus, fibroblasts, endothelial cells and the tumor cells themselves can respond to these initial stimuli with changes in their phenotypic and functional profiles. In turn, these cells, through a network of cell-to-cell interactions as well as via the secretion of chemokines, cytokines, growth factors and enzymes contribute to the establishment of an unique tumor microenvironment.[2] Besides favoring tumor progression and tissue invasion, such a microenvironment can modulate antitumor immune responses by releasing immunosuppressive factors and/or by facilitating the accumulation/development of regulatory cells, such as tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), FOXP3$^+$ regulatory T cells (Tregs) and tolerogenic dendritic cells (DCs).[1,3] Remarkably, most immunosuppressive factors that are produced in the tumor microenvironment, such as transforming growth factor β (TGFβ), indoleamine 2,3-dioxygenase (IDO) and prostaglandin E2 (PGE_2), also proved effective in inhibiting natural killer (NK) cell function, through the downregulation of several activating NK receptors that contribute to tumor-cell recognition and killing.[4,5]

Despite these immunosuppressive mechanisms, the immune system plays a role in the host defense against tumors. The clinical relevance of local immune responses to human cancer was brought up by a series of recent reports.[3] In addition, different studies on animal models suggest that immune cells, in particular NK cells, can control or even block the early steps of tumor development.[6] This implies that tumors reaching a critical size or stage of development have eluded immunosurveillance or biased the equilibrium with the host defenses. The latter situation depends on the proliferation rate and/or the immunogenicity of tumor cells as well as on the capacity of tumor nests of limiting infiltration by immune effector cells.

When tumor cells are not promptly eliminated, they may adapt to the pressure exerted by immune effector cells and find a way to escape the host immunosurveillance. This may occur through modifications of the tumor cell properties (e.g., under the influence of the tumor microenvironment) or through the selection of clones displaying high proliferation rates and/or low immunogenicity. This process, defined as "tumor immunoediting," may play a central role in the disruption of the initial equilibrium between the host and the tumor. In addition, it may be relevant during later steps of tumor development and metastatic spread.[1,6]

In a recent study,[7] we described a novel mechanism of immunoediting by which melanoma cells acquire resistance to interleukin (IL)-2-activated NK cells (**Fig. 1**). We first observed that, in primary melanoma lesions, there is a rather limited NK cell infiltration. Based on this finding, we attempted to reproduce this situation in vitro. To this end, we established melanoma-NK cell co-cultures at E/T ratios reflecting NK cell infiltrates (i.e., low NK/tumor cell ratios). We found that, after an initial tumor cell killing, NK cells and residual melanoma cells persisted in equilibrium for several days. In line with this finding, we observed that, after 2 days of co-culture, melanoma cells increased the expression of (both classical and non-classical) HLA-I molecules at the cell surface, and became resistant to NK cell-mediated killing. Increased HLA-1 expression was consequent to the release of interferon γ (IFNγ) by NK cells upon interaction with melanoma cells. Moreover, the level of IFNγ was proportional to the strength of melanoma-induced NK-cell stimulation. Since the acquisition of the resistant phenotype on melanoma cells inversely correlated with the magnitude of the initial NK-mediated

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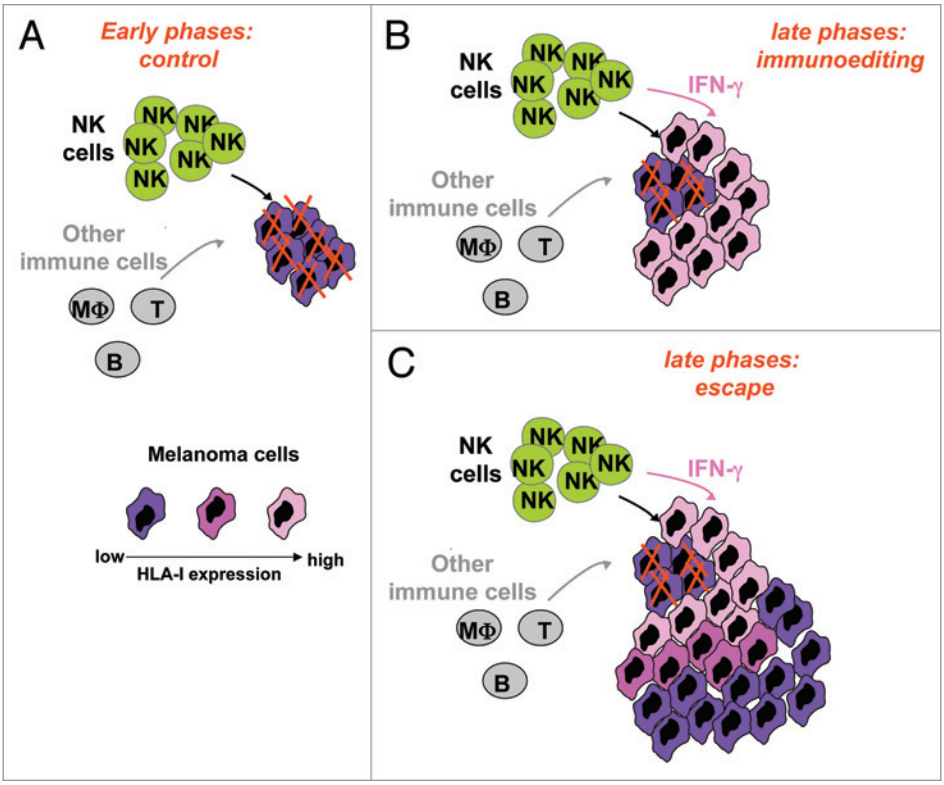

Figure 1. Schematic representation of NK cell-mediated melanoma immunoediting. (**A**) Natural killer (NK) cells, together with other immune cells, can control the early phases of tumor growth. (**B**) In lesions that have grown beyond a defined size or have limited NK cell infiltration, NK cells may be unable to completely eliminate tumor cells. Under the stimulus of inflammatory cytokines and the interaction with target cells, NK cells produce interferon γ (IFNγ), which, in turn, induces HLA-I upregulation on melanoma cells. (**C**) Proliferation of NK cell-resistant melanoma cells results in further decreased NK/melanoma cells ratios. In these conditions, killing of melanoma cells is further limited, while the IFNγ-dependent HLA-I upregulation remains confined to a limited number of melanoma cells.

tumor cell lysis, enhancement of the NK cell cytolytic potential (e.g., by exposure to IL-15), could partially overcome melanoma cell resistance.

Interestingly, the upregulation of HLA-I expression on tumor cells, while favoring resistance to NK-mediated killing, may increase the sensitivity of melanoma cells to T-cell responses. Although this may hold true for classical HLA-Class I molecules, the parallel upregulation of non-classical HLA-I molecules (as it occurred in our experimental setting) may negatively affect T-cell responses.[8] In addition, as suggested by our histological analyses of primary melanoma specimens, HLA-I upregulation may actually be confined to melanoma cells proximal to NK cells. Finally, melanoma cells, in response to IFNγ stimulation, may also produce immunosuppressive factors, including IDO and PGE_2, which are capable of interfering with lymphocyte activity.[4]

In conclusion, a number of experimental evidences support the notion that NK cells may represent a promising tool for the successful therapy of both hematological and solid tumors.[9,10] However, an effective NK cell-based immunotherapeutic strategy is still challenged by the possible modulation of NK-cell function in vivo, and by changes in tumor cells favoring the escape from NK cell-mediated recognition and killing. In the latter scenario the results of our study suggest that an appropriate NK cell stimulation (e.g., by culture in the presence of IL-15) may allow NK cells to overcome some tumor escape mechanisms occurring locally, at tumor sites. Our results also emphasize that high number of NK cells should reach the tumor to mediate its eradication and to prevent the survival of resistant cancer cells. Future studies focused on the regulation of NK-cell migration to the tumor, with particular attention on the functional interplay between NK cells, fibroblasts[5] (which often surround tumor nests) and tumor-associated endothelial cells, should provide important clues to improve NK cell-based immunotherapies.

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