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Regulation of NKG2D signaling during the epithelial-to-mesenchymal transition

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Abbreviations: CRC, colorectal carcinoma; EMT, epithelial-to-mesenchymal transition; NK, natural killer; MIC, MHC class I polypeptide-related sequence; NKG2D, natural killer group 2 member D; ULBP, UL16-binding protein

The plasma membrane receptor natural killer group 2 member D (NKG2D) underpins a major mechanism whereby natural killer (NK) and T cells recognize malignant cells. We have recently demonstrated that the epithelial-to-mesenchymal transition, one of the first steps of metastatic dissemination, is under the control of an immunological checkpoint that relies on NKG2D-mediated immune responses.

Natural killer (NK) cells play a major role in cancer immunosurveillance. Unlike T lymphocytes, NK cells lack antigen-specific receptors. Nonetheless, NK cells are able to detect malignant cells by monitoring the surface of the latter for the expression of ligands for their own inhibitory and activatory receptors. In particular, inhibitory receptors are sensitive to the expression of self proteins, mainly MHC class I molecules, hence impeding the unwarranted activation of NK cells. Thus, NK cells can complement T cell-mediated antitumor responses by eliminating malignant cells that evade the T-cell cytotoxicity through the loss of MHC class I molecules. Conversely, a distinct set of NK-cell receptors deliver activatory signals upon binding to stress-induced self proteins. Killer cell lectin-like receptor subfamily K, member 1 (KLRK1), best known as natural killer group 2 member D (NKG2D) is an activating receptor expressed by both NK cells and T cells that provides a major contribution to cancer immunosurveillance.[1] NKG2D is sensitive to MHC class I polypeptide-related sequence (MIC)A/B and UL16 binding protein(ULBP)1–6 molecules, all of which are upregulated in response to cellular stress, infection and transformation.[2] Thus, NK cells are capable of recognizing cells that express on their surface a broad panel of stress-inducible proteins. However, the immune system is often unable to completely eradicate malignant cells, but rather eliminates only the most immunogenic ones. This situation, which is known as "immunoediting," results in the selection of cancer cells that are able to evade the immune response. Thus, in the course of tumor progression, malignant cells progressively become resistant to NKG2D-mediated immunity.[2]

We have recently investigated the role of NKG2D signaling in colorectal carcinoma (CRC),[3] finding that specific NKG2D ligands (NKG2DLs), i.e., MICA, MICB and ULBP1, are expressed in relevant amounts on the surface of healthy colon epithelial cells.[3,4] This observation argues against the "induced" or "stressed-self" hypothesis, postulating that NKG2DLs are not expressed by benign cells. As many other groups had detected NKG2DL expression in several healthy tissues, including the gastrointestinal epithelium, we asked why healthy colorectal epithelial cells are not actively eliminated by the immune system. We found that MICA/B display an apical distribution on epithelial cells, and we were unable to obtain evidence of a physical interaction between these proteins and NKG2D, which is expressed by intraepithelial lymphocytes located on the basal side of the epithelium and in the lamina propria (**Fig. 1**). Thus, the maintenance of epithelial integrity and polarity appears to protect epithelial cells from being eliminated upon the interaction of NKG2D with its ligands.

The first steps of the metastatic cascade of malignant carcinoma cells involve dramatic morphological and genetic changes, resulting in the loss of epithelial characteristics coupled to the gain of mesenchymal properties. This program, which is frequently associated with the acquisition of invasive and migratory properties by cancer cells, is generally referred to as "epithelial-to-mesenchymal transition" (EMT).[5] Our observations suggested that the loss of polarization of malignant epithelial cells during the EMT may allow for the interaction of NKG2DL with NKG2D-expressing immune effectors, resulting in the activation of an antitumor immune response. To test this hypothesis, we analyzed whether the expression of NKG2D and NKG2DLs in CRC correlated with the presence of EMT characteristics. We observed that highly differentiated lesions, which retained epithelial features (e.g.,

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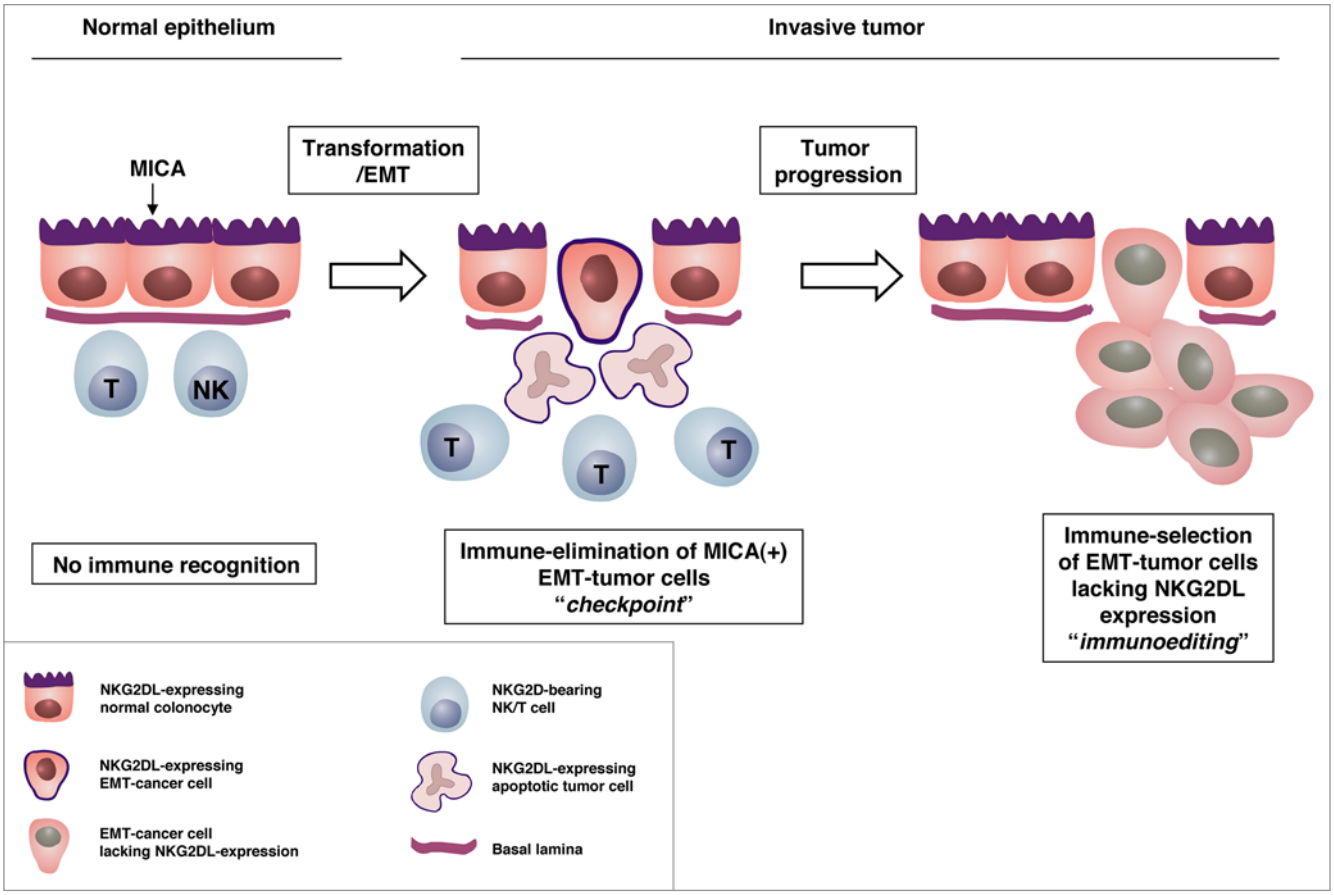

Figure 1. NKG2D-mediated immune response during the epithelial-to-mesenchymal transition. MHC class I polypeptide-related sequence (MIC)A/B are expressed on the apical surface of healthy colonocytes, and there is no evidence of a physical interaction between these molecules and natural killer group 2 member D (NKG2D), which is expressed by lymphocytes infiltrating the basal side of the epithelium and the lamina propria. This prevents the recognition of healthy cells by natural killer (NK) cells and hence the development of autoimmune responses. The expression of NKG2D ligands (NKG2DLs) is increased on the surface of malignant cells. Moreover, along with the loss of epithelial integrity and polarity that accompanies the so-called "epithelial-to-mesenchymal transition" (EMT), MICA/B can diffuse all over the plasma membrane of mesenchymal cells, leading to their elimination by NK cells. Only malignant cells that evolve mechanisms to circumvent NKG2D-mediated immune responses, such as the repression of NKG2DLs, are able to establish advanced lesions and metastases.

the expression of E-cadherin), expressed MICA/B in a polarized, apical manner. Such an expression pattern was lost in advanced CRCs, demonstrating that CRC progression might be accompanied by the loss of MICA/B polarization that characterizes the healthy epithelium.[3] Thus, as a consequence of the EMT-related loss in epithelial integrity and polarity, MICA/B might diffuse along the membrane of mesenchymal cells. This model suggests that, in the course of EMT, carcinoma cells might become susceptible to recognition and elimination by NKG2D-expressing lymphocytes. In line with this hypothesis, a dramatic increase in the number of NKG2D$^+$ cytotoxic tumor-infiltrating T lymphocytes (TILs) was observed in advanced CRC specimens, notably among lesions lacking MICA/B expression. Moreover, different maneuvers that promote the EMT, such as the expression of snail family zinc finger 1 (SNAI1), the inhibition of glycogen synthase kinase 3β (GSK3β), or the administration of transforming growth factor β (TGFβ), stimulated the expression of NKG2DL by several cancer cell lines, rendering these cells more susceptible to NK cell-mediated killing.[3] The EMT-associated loss of E-cadherin expression might also constitute a target for anticancer immune responses, as E-cadherin is the ligand of the inhibitory NK-cell receptor killer cell lectin-like receptor subfamily G, member 1 (KLRG1).[10] Of note, we and others have previously shown that the transcription factors SP1 and SP3 are essential regulators of NKG2DL expression in cancer cells.[6,7] Our recent study demonstrates a prominent role for SP1 in the upregulation of NKG2DL by CRC cells undergoing the EMT, confirming the relevance of SP transcription factors in the control of NKG2DL expression in transformed cells.

Collectively, our results indicate that the EMT regulates NKG2D-mediated anticancer immune responses, favoring the development of malignant cells expressing low levels of NKG2DLs and other activating mechanisms of NKG2D evasion. Consistent with our results, a link between the EMT and immunoediting in vivo has previously been demonstrated. Thus, the transplantation of epithelial cancers expressing the *neu* oncogene into syngeneic mice induced

an immune response leading to apparent tumor eradication. However, these mice subsequently relapsed following the growth of lesions enriched in Neu$^-$ malignant cells, which acquired a mesenchymal phenotype.[8] These findings suggest that the EMT-associated immunoediting favors the establishment of several mechanisms whereby malignant cells can evade NKG2D-mediated immune responses, including the epigenetic repression of NKG2DL and the shedding of soluble MICA.[4,9] Thus, an NKG2D-dependent immunological checkpoint may be in place to control the malignant progression of carcinoma cells through the EMT.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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