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The wolf in sheep's clothing

Platelet-derived "pseudo self" impairs cancer cell "missing self" recognition by NK cells

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Abbreviations: MHC, major histocompatibility complex; NK, natural killer; TGF, transforming growth factor

Metastasis is strongly inhibited in thrombocytopenic mice. This phenotype is reversed by NK cell depletion which indicates that platelets may facilitate tumor progression and metastasis by interfering with NK cell immunosurveillance. Understanding the underlying mechanisms may help us to reinforce anti-tumor immunity and NK-based immunotherapy in cancer patients.

NK cells are cytotoxic lymphocytes, which play a major role in anti-tumor immunity and can prevent tumor progression and metastasis. Their reactivity is guided by the principles of "missing self" and "induced self" recognition, which imply that cells with low or absent expression of MHC class I ("missing self") and/or expression of stress-induced ligands for activating NK receptors ("induced self") are preferentially recognized and eliminated by NK cells. In addition, reciprocal interactions of NK cells with other hematopoietic cells such as dendritic cells have been described to result in altered reactivity of both involved cell types.[1] The interplay of NK cells with platelets was by far less studied, although the latter have been known for many years to facilitate tumor progression and metastasis. Platelets may influence tumor cells through multiple mechanisms including release of growth factors that stimulate tumor proliferation and neoangiogenesis or by facilitating vessel wall penetration thereby opening metastasizing cells in the blood the door to their metastatic niche.[2] More recently, their immunomodulatory properties are increasingly being recognized.[3] In mice, there is little to no metastasis in the absence of platelets, and this is reversed by additional depletion of NK cells.[4] Thus, inhibition of NK reactivity may be crucial for the metastasis-promoting effects of platelets. However, besides mechanistic hypotheses proposing that tumor cells may "hide behind" platelets thereby preventing access of immune cells, the molecular mechanisms influencing platelet-tumor-NK cell interaction were largely unclear.[2]

In our recent work[5] we unravel transfer of MHC class I to malignant cells by platelets as a mechanism whereby (metastasizing) tumor cells "hide" from "missing self" recognition by NK cells. Various flow cytometric and microscopic techniques including confocal and immuno-electron microscopy revealed that constitutively MHC Class I-negative/low tumor cells acquire MHC Class I upon interaction with platelets. Analyses with allotype-specific antibodies confirmed that MHC class I was in fact transferred to the tumor cells and excluded that platelets merely induced upregulation of the tumor cells' own MHC. NK reactivity was impaired in cultures with tumor cells that displayed MHC Class I derived from platelets of the NK cell donor. Blocking MHC Class I restored NK reactivity under this condition, while responses to tumor cells that had not been exposed to platelets were not altered.

Our findings indicate how (metastasizing) tumor cells may downregulate MHC Class I to evade T-cell anti-tumor reactivity[6] without becoming prone to NK immunosurveillance due to an immunophenotype of false pretenses: the transfer of platelet-derived MHC Class I molecules that present "unsuspicious" peptides reflecting the normal ligandome of the megakaryocyte lineage would not stimulate T-cell responses but result in an NK-inhibitory "pseudo-self" phenotype. It should be noted that beyond prevention of "missing self" detection by platelet-derived MHC Class I, multiple other immunomodulatory ligands for receptors expressed on NK cells can be transferred by platelets and certainly also influence NK anti-tumor immunity.[7] The picture becomes even more complex when we consider that platelets may also influence

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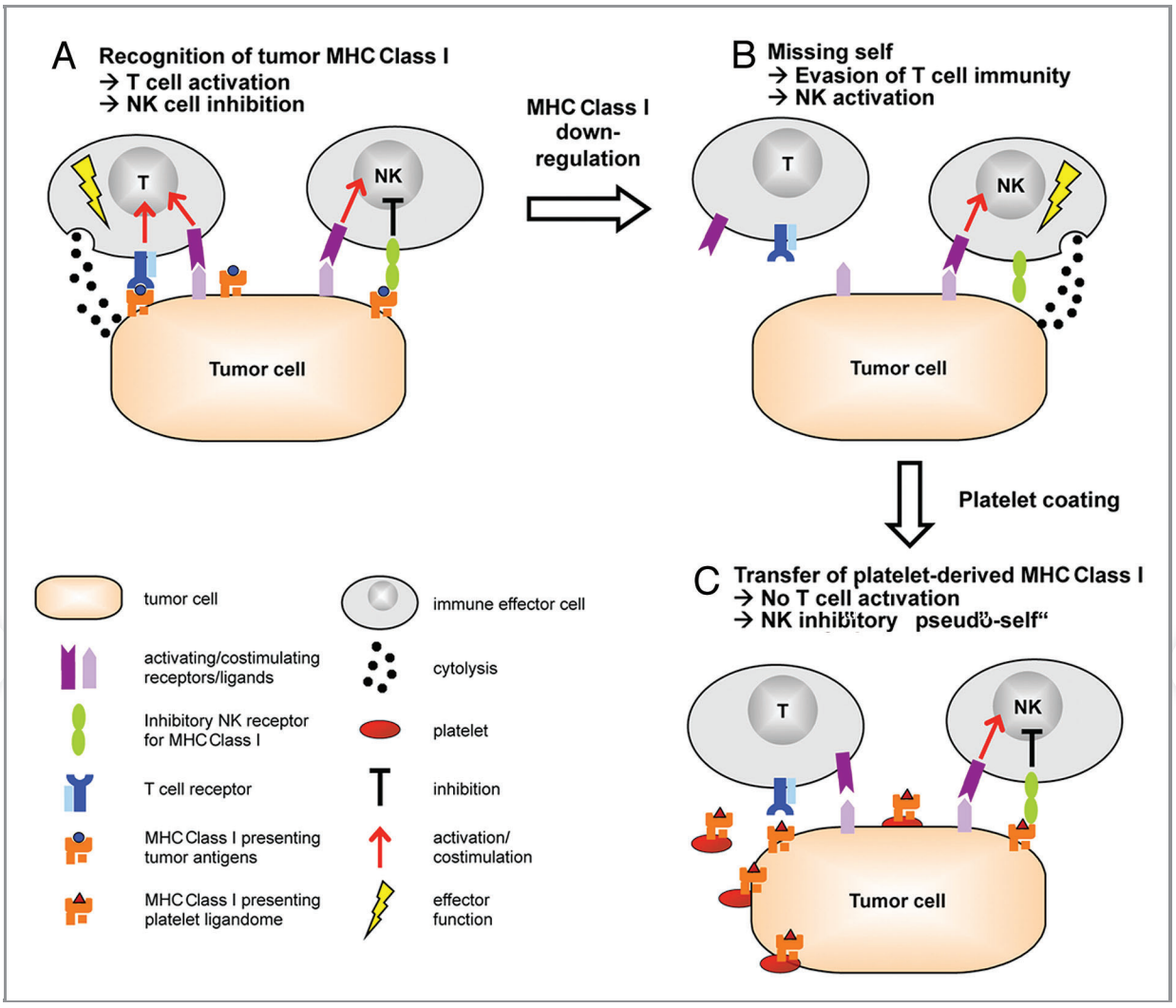

Figure 1. The role of platelet-derived "pseudo-self" in tumor immune evasion. (A) T cell immunity is mediated by recognition of tumor antigens presented by the tumor cell's own MHC Class I; the latter at the same time inhibits NK cell reactivity. (B) MHC Class I downregulation enables escape from T cell immunity but renders tumor cells susceptible to NK "missing self" recognition. (C) Transfer of platelet MHC Class I results in a "pseudo-self" phenotype which impairs NK immunosurveillance by concealing the "missing self" without inducing T cell reactivity due to presentation of "unsuspicious" peptides.

the reactivity of NK cells by soluble factors that are released upon tumor cell-platelet interaction. This comprises secretion of TGFβ, which causes downregulation of NKG2D on NK cells. NKG2D plays a prototypical role in NK "induced self" recognition, and reduction of its expression by platelet releasate results in impaired reactivity against NKG2D ligand-expressing tumor targets.[8] Again, multiple other platelet-derived cytokines and growth factors certainly also play a role.[3,7]

Together, current data indicate that platelets influence NK cells by multiple different mechanisms. This may also explain discrepancies between our results and e.g., the findings of Nieswandt et al. in the murine system, who found that platelets impaired NK cytotoxicity by a mechanism independent from MHC Class I in their experimental setting.[4] In line with the concept that NK cell responses are governed by a balance of signals mediated by multiple activating and inhibitory receptors,[1] the available data show that platelets influence NK cell anti-tumor reactivity by various mechanisms including impaired "induced self" and "missing self" recognition with multiple soluble and membrane-bound molecules being involved. Since platelets also affect immune effector cells other than NK cells, promote metastasis by multiple mechanisms beyond influencing anti-tumor immunity, and because metastatic tumor spread is also influenced by the plasmatic coagulation system,[7] dissection of specific tumor-promoting effects mediated by platelets is challenging. This especially holds true for animal models, where species-specific differences between mice and men with regard to the function of immunomodulatory molecules (including those expressed by platelets) may additionally come into play.[2,7] Thus, while mouse models have largely added to our understanding of the role of platelets in immunity in the past and will certainly

do so in the future, the understanding of NK-evasion mechanisms mediated by platelets is well aided by rational in vitro and ex vivo analyses. Our recent publication may serve well to exemplify such approaches.

The trilateral crosstalk of NK cells, platelets, and tumor cells in cancer progression and metastasis obviously constitutes a complex mechanism, which we presently are only beginning to understand. Notably, data from mouse models utilizing inhibitors of platelet aggregation indicate that adhesion (potentially resulting in transfer of immunoregulatory molecules) may be more relevant than aggregation (associated with release of soluble factors) for enabling tumor cell dissemination.[9] This underlines the relevance of the transfer of immunoregulatory molecules between platelets and tumor cells as described in our study,[5] but also has important implications for pharmacological approaches aiming to prevent metastasis-enhancing platelet effects.[2,7] As numerous attempts are presently being made to introduce NK cells in the treatment of cancer,[10] we are convinced that a better understanding of how platelets contribute to the evasion of tumor cells from NK anti-tumor reactivity holds promise to identify strategies that ultimately may improve immunotherapeutic options for cancer patients.

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