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KLRG1+ natural killer cells protect against pulmonary metastatic disease by immunosurveillance

Philipp Renner[1], Jordi Rovira[2], Christoph Klein[3], Hans-Jürgen Schlitt[1], Edward K Geissler[1], and Alexander Kroemer[1,*]

[1]Department of Surgery; University Hospital Regensburg; University of Regensburg; Regensburg, Germany; [2]Department of Nephrology and Renal Transplantation; Hospital Clinic de Barcelona; Barcelona, Spain; [3]Experimental Medicine and Therapy Research; University of Regensburg; Regensburg, Germany

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Natural killer cells (NK cells) play a key role in cancer immunosurveillance. However, their activity is highly dependent upon their maturation stage, which in turn relates to organ distribution. Here, we discuss the role of intrinsic master transcription factors and extrinsic IL-15 signaling on NK cell-mediated immune protection against murine pulmonary metastasis.

One of the most intriguing questions in cancer immunology is how cancer cells escape immunosurveillance. Considering the course of most malignancies, cancer cells escape immune regulation at least twice. The first occurrence is when healthy cells transform into malignant cells without being destroyed immediately, and the second takes place when some of these cancer cells leave their origin to successfully colonize distant organs.

Natural killer (NK) cells are considered potent sentinels for cancer, and their relevance has been confirmed in several studies. In experimental models, NK cells protect mice against tumors arising from adoptively transferred cancer cells or tumors induced by chemical carcinogens. In humans, NK cell infiltration into tumor tissue is generally associated with a better clinical outcome, whereas suppressed NK cell activity can be a negative prognostic factor for cancer development or disease progression.[1] However, the NK cell compartment does not consist of a homogeneous cell population, but rather of phenotypically and functionally distinct subsets. We, and others, have recently found that expression of the maturation markers CD27 (a member of the TNF-receptor superfamily) and CD11b, as well as KLRG1 (the killer cell lectin-like receptor subfamily G, member 1), allows for the discrimination of murine NK cell subpopulations with differing levels of antitumor activity.[2,3]

The expression of CD27 and KLRG1 are regulated by cell-intrinsic pathways integral to the NK cell's differentiation status. This differentiation process is controlled through expression of the T-box transcription factors T-bet (Tbx21) and Eomes (eomesodermin).[4] Importantly, we observed that T-bet deficient mice lack $CD27^{lo}KLRG1^{+}$ NK cells that underlies a loss of protection against pulmonary colonization after tail vein injection of CT26 colorectal cancer cells (**Fig. 1**).[5] Considering that T-bet also plays an important role in regulating the fate and activity of T cells, we also evaluated whether T cell alterations were a contributing factor and determined that $Rag1^{-/-}$ mice lacking T and B cells were protected against CT26 pulmonary metastasis, whereas $Rag1^{-/-}T\text{-}bet^{-/-}$ showed extensive colonization of tumor cells in the lung. The importance of $CD27^{lo}KLRG1^{+}$ NK cells to the prevention of metastatic disease was further clarified in adoptive transfer experiments, in which antitumor protection could be restored in T-bet deficient mice by injection of T-bet competent $CD27^{lo}KLRG1^{+}$ NK cells (**Fig. 1**). Interestingly, immunosurveillance in $T\text{-}bet^{-/-}$ mice could also be recovered by reconstitution with wild-type $CD27^{hi}KLRG1^{-}$ NK cells that became $CD27^{lo}KLRG1^{+}$ upon adoptive transfer in vivo. Our findings revealing the essential role of T-bet in cancer immunosurveillance is in line with published data by other groups, who found that T-bet deficiency is also associated with an augmented tumor-burden in a B16.F10 melanoma model and an increased rate of metastases in the TRAMP prostate cancer model.[6,7]

To compensate for the immaturity of T-bet-deficient NK cells and, in premise, thereby improve their anticancer activity, we applied trans-presented recombinant IL-15, the ectodomain of the mouse IL-15Rα - chain fused to the Fc domain of human IgG1 (rIL-15/IL-15Rα/Fc),[8] to T-bet deficient animals. It is known that NK cells are highly responsive to common-γ chain cytokines.[9] For example, NK cells stimulated with trans-presented IL-15 in vivo overexpress the stimulatory receptor killer cell lectin-like receptor K1 (KLRK1, better known as NKG2D), as well as effector molecules (e.g., granzyme B, perforin) that mediate tumor

*Correspondence to: Alexander Kroemer; Email: alexander.kroemer@ukr.de
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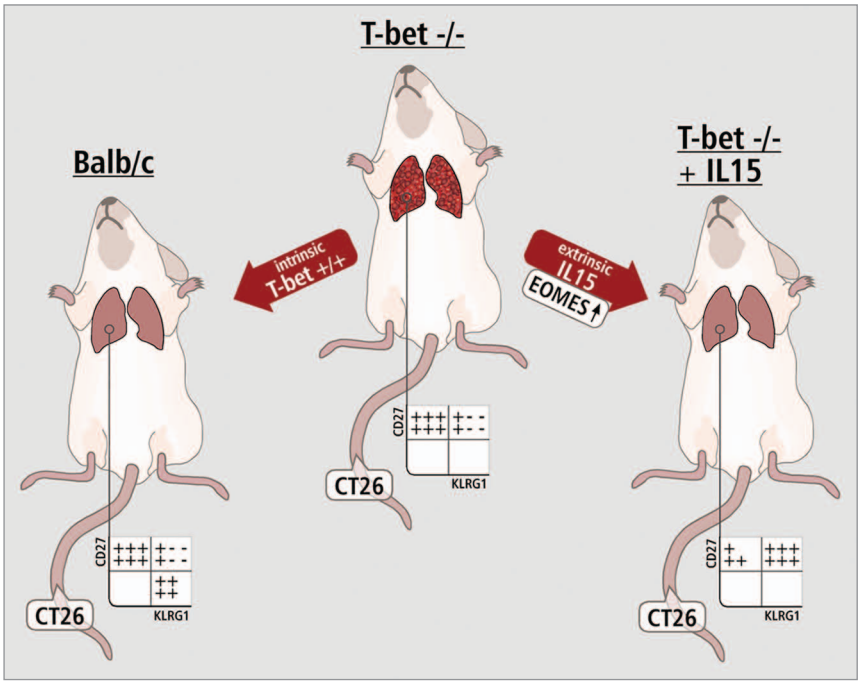

Figure 1. Distribution of natural killer (NK) cell subsets with distinct anticancer properties in a metastatic disease model. Natural killer (NK) cells with tumor-inhibitory activities are $CD27^{lo}KLRG1^{+}$ and dependent upon the T-box transcription factors T-bet and Eomes. CT26 colorectal carcinoma cells were injected intravenously into $T\text{-}bet^{-/-}$ mice (center), Balb/c wild-type mice (left), and $T\text{-}bet^{-/-}$ mice treated with IL-15 (right) and monitored for metastatic burden and heterogeneous NK cell subsets. Eomes, eomesodermin; KLRG1, killer cell lectin-like receptor subfamily G, member 1; T-bet, T box 21.

immunosurveillance.[8] Indeed, after treating $T\text{-}bet^{-/-}$ mice with IL-15, animals were protected from lung colonization by adoptively transferred CT26 carcinoma cells with an efficiency similar to that of their T-bet competent littermates (**Fig. 1**). This effect was accompanied by rapid in vivo expansion of NK cells and upregulation of Eomes, as well as KLRG1. These observations make IL-15 an interesting treatment option for immunotherapies in cancer patients. In fact, the first clinical trials are currently being conducted to evaluate the potency of IL-15 in the clinical setting (e.g., http://www.clinicaltrials.gov: NCT01021059, NCT01369888, NCT01189383, and NCT01337544).

To date, it can be argued that insufficient emphasis has been placed on experiments targeting causal factors driving the very early spread of cancer cells,[10] some of which may survive and progress to metastases that account for most cancer deaths. In this respect NK cell subpopulations may be critical to the development of early metastases not only because of their effector functions, but also due to their differential distribution in specific organs. For instance, $CD27^{hi}$ NK cells in mice predominate in bone marrow and lymph nodes, and rapidly respond to IL-15 stimulation as a result of their high expression levels of IL-15 receptor-β. Conversely, $CD27^{lo}$ NK cells predominate in extra-lymphoid organs such as lungs and are more tightly regulated (relative to $CD27^{hi}$ cells) in terms of effector functions because of their relatively low IL-15 receptor-β levels and high NK inhibitory receptor expression.[2] We are currently exploring whether this NK cell heterogeneity results in distinct tissue-specific NK cell-mediated antitumor responses, thereby impacting the pattern and early onset of metastatic disease.

Further research is clearly required to elucidate the regulation of NK cell subpopulation homing, effector function, and anticancer properties, especially in relation to the expression of the T-box transcription factors T-bet and Eomes, as well as in regards to the influence of exogenous IL-15. We believe that new studies should emphasize the use of spontaneous tumor models so that the role of NK cell subsets in the genesis of metastatic disease can be better understood, perhaps leading to novel therapeutic strategies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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