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ATM activation mediates anticancer immunosurveillance by natural killer and T cells

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The DNA damage response (DDR), which is frequently activated in cancer cells, has been proposed to operate as an early barrier against oncogenesis. We have recently shown that ATM mediates the spontaneous regression of Eμ-*myc*-driven murine B-cell leukemia in a natural killer and T cell-dependent manner. The DDR partially enhanced immune recognition by stimulating the expression of the DNAM-1 ligand CD155.

The DNA damage response (DDR) is activated early in precancerous breast, colon, lung and prostate lesions and was suggested to operate as a barrier against oncogenesis by virtue of its ability to trigger cell senescence or apoptosis.[1] The activation of oncogenes in precancerous cells often leads to collapsed replication forks and DNA breaks that are detected by the phosphoinositide-3-kinase-related kinases ataxia telangiectasia, mutated (ATM) and ATM-and Rad3-related (ATR), which initiate the DDR. In particular, ATM senses double-strand DNA breaks while ATR detects single-strand DNA at stalled and collapsed replication forks. ATM and ATR directly activate a number of signal transducers including the serine/threonine checkpoint kinases 1 and 2 (CHK1 and CHK2). As a consequence, these kinases phosphorylate and regulate several effector proteins including, breast cancer 1, early onset (BRCA1), the E2F transcription factor 1 (E2F1), the phosphatase cell division cycle 25C (CDC25) and multiple p53 family members. The resulting cell cycle arrest allows time for the repair of DNA lesion before replication resumes. If the DNA damage is irreparable, p53 family members trigger cell senescence (an irreversible cell cycle arrest) or apoptotic cell death. The constitutive activation of the DDR in precancerous cells is thought to underpin the selection and emergence of cells in which the function of DDR regulators or effectors is lost. This often affects p53, which is one of the most frequently mutated or functionally inactivated genes in human tumors.

Besides arresting the cell cycle and activating apoptosis (two cell-intrinsic effects), the DDR can also alert the immune system to the danger posed by DNA lesions by stimulating the expression of damage-associated molecular pattern molecules (DAMPs), chemokines or ligands for activating immune receptors such as NKG2D and DNAM-1.[2] A role for the immune system in the oncosuppressive functions of the DDR was suggested by the enhanced susceptibility of DNAM-1- and NKG2D-deficient mice to tumorigenesis.[3] Importantly, p53 is dispensable for the induction of murine NKG2D and DNAM-1 ligands in response to DNA damage.[4] A report by Xue et al. suggested that the activation of p53 leads to the recruitment of innate immune cells to the tumor bed.[5] However, the role of p53 in the recruitment of immune cells by malignant cells that express ligands for activating immune receptors remains to be elucidated.

At present, little is known on the links between the DDR, and anticancer immunosurveillance in humans. Consistent with data obtained in murine models, genotoxic agents upregulate DNAM-1 and NKG2D ligands on the surface of multiple myeloma and Ewing's sarcoma cells.[6] Furthermore, a number of studies have shown that the expression of NKG2D ligands on the surface of cancer cells or the presence of soluble NKG2D ligands in the serum correlates with disease progression in patients affected by various tumor types.

To test whether oncogene induced DNA damage initiates an antitumor immune response in vivo, we performed a longitudinal study of tumor load in Eμ-*myc* transgenic mice.[7] Similar to many human Burkitt lymphomas, Eμ-*myc* mice overexpress *Myc* under the control of the immunoglobulin heavy chain enhancer region (Eμ). Replication stress as a result of Myc overexpression was shown to induce DNA damage in both preneoplastic and malignant tumor cells of Eμ-*myc* mice. We found that peripheral early-stage B lymphomas in Eμ-*myc* mice spontaneously regressed between 41 and 65 days of age. The administration of the ATM inhibitor KU55933 prior to 41 days of age impaired tumor regression, demonstrating that ATM plays a critical role in this process. ATM-mediated tumor regression critically depended on $CD4^+$, $CD8^+$ and natural killer (NK) cells, as it was significantly impaired in mice that had received T or NK cell-depleting antibodies (**Fig. 1**).[7] In agreement with a role for

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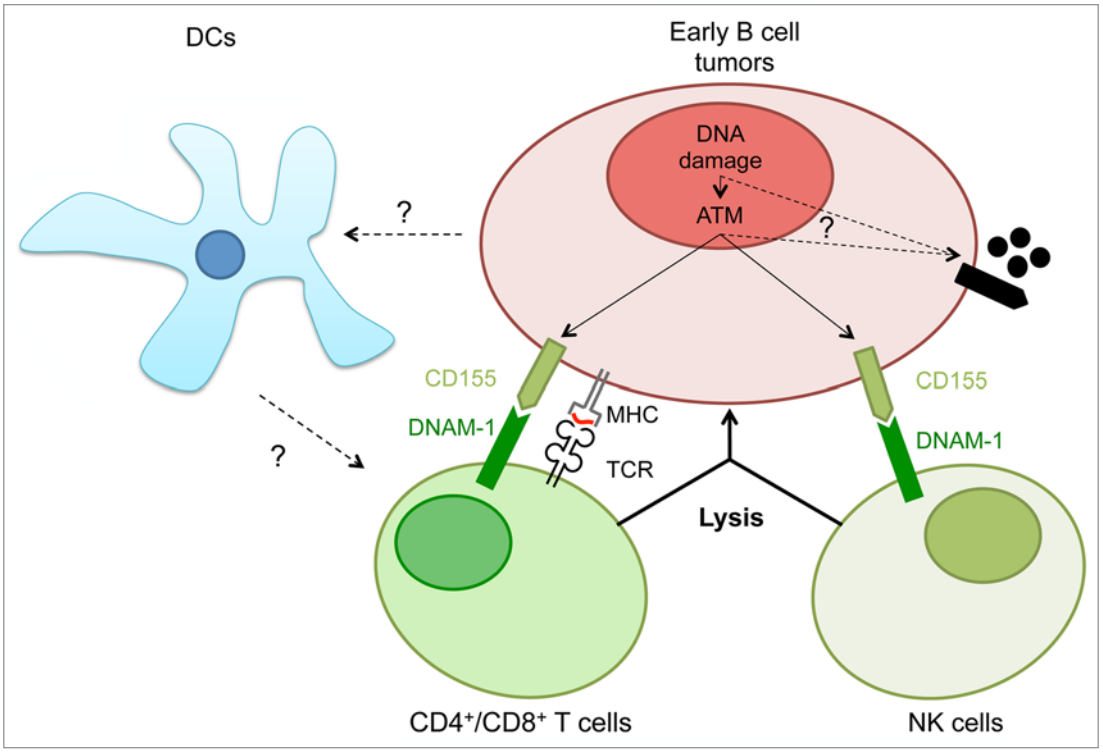

Figure 1. Immunosurveillance of early B-cell lymphomas in Eμ-*myc* mice. Oncogenic stress-dependent DNA damage activates the DNA damage sensor ATM, leading to the upregulation of CD155 and possibly other immunostimulatory molecules on the surface of (pre)cancerous cells. These immunomodulatory surface molecules drive the attack and lysis of cancer cells by natural killer (NK) cells. Tumor-associated antigens presented by cancer cells and dendritic cells (DCs) activate T cells to contribute to tumor eradication in the periphery.

T cells in this setting, the blood of Eμ-*myc* mice contained T cells with an activated phenotype prior to tumor regression.

Interestingly, the levels of MHC Class I H-2K^b molecules on the surface of malignant cells decreased during tumor regression, suggesting that H-2K^b may present tumor-associated antigens to T cells. It is also possible that reduced H-2K^b levels enabled "missing-self recognition" of cancer cells by NK cells. The DNAM-1 ligand CD155 was upregulated and exposed on the surface of malignant cells in an ATM-dependent manner, and the blockage of DNAM-1 impaired tumor regression. These findings suggest that "induced-self recognition" also contributes to the eradication of cancer cells in this model. NKG2D ligands, which have previously been implicated in anticancer immunosurveillance in Eμ-*myc* mice, were not detected on the surface of neoplastic cells prior to regression, consistent with previous studies.[3] However, NKG2D ligands were expressed by cancer cells post-regression, indicating that the NKG2D system may prevent disease relapse. Tumors appearing after 65 d of age often correlated with reduced levels of circulating NK and T cells, possibly owing to the increased abundance of cancer cells within the bone marrow and the loss of common lymphocyte progenitors. In addition, in these animals, DNAM-1 expression was decreased on NK cells but enhanced on a subset of CD4$^+$ T cells. Low expression of DNAM-1 presumably impairs the response of NK cells to DNAM-1 ligands, which may contribute to the ability of cancer cells to evade NK-mediated immunosurveillance.[8] The function of DNAM-1$^+$CD4$^+$ T cells, which also express CD25, in immune surveillance is currently unclear. However, a recent report suggests that regulatory T cells (Tregs) express T-cell immunoreceptor with Ig and ITIM domains (TIGIT), an inhibitory receptor that binds CD155.[9] It is therefore possible that the expression of DNAM-1 on the surface of Tregs contributes to their activation and hence to the suppression of antitumor immune responses. It remains to be seen whether the NK and T cells of Burkitt lymphoma patients show similar DNAM-1 expression profiles.

In summary, our data suggest that Eμ-*myc* mice provide a valuable model to study spontaneous tumor regression and immunosurveillance. Future studies in may shed light on the mechanisms leading to spontaneous tumor regression in humans, which have been difficult to study so far owing to their rare incidence. A better understanding of spontaneous tumor regression may be helpful in designing regimens that successfully reinstate immunosurveillance in cancer patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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