CANCER

Unleashing NK cell anti-metastatic activity

> LDC1267 was well tolerated and reduced metastasis in mouse models of melanoma and breast cancer

Metastasis is the most lethal aspect of cancer. Writing in *Nature*, Penninger and colleagues now demonstrate that natural killer (NK) cells have an important role in controlling this process, and that their activity is limited by negative regulators including the ubiquitin ligase CBL-B (casitas B-lineage lymphoma B). Moreover, they report the development of a small-molecule inhibitor of an upstream activator of CBL-B that appears to confer a 'licence to kill' to NK cells, showing anti-metastatic activity in several mouse models of cancer.

CBL-B is known to negatively regulate the anticancer activity of $CD8^+$ T-cells. However, it was found that mice lacking *Cbl-b* (*Cbl-b*$^{-/-}$ mice), as well as mice in which the E3 ligase function of CBL-B was inactivated owing to the C373A mutation (*C373A*$^{KI/KI}$ mice), had enhanced anticancer responses even in the absence of adaptive immune cells, indicating that CBL-B also suppresses the activity of innate immune cells. Immunohistochemistry pointed to NK cells, which were found to be enriched in tumours of *Cbl-b*$^{-/-}$ mice.

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Ensuing *in vitro* experiments demonstrated that NK cells from *Cbl-b*$^{-/-}$ and *C373A*$^{KI/KI}$ mice are more cytotoxic than wild-type NK cells, and in a mouse model of metastatic melanoma, *Cbl-b*$^{-/-}$ and *C373A*$^{KI/KI}$ mice had fewer metastatic lesions than their wild-type counterparts. Depletion or functional inactivation of NK cells abolished the enhanced anti-metastatic responses of these mice. Using NK cell adoptive transfer in mouse models of metastatic melanoma and mammary carcinoma, wild-type mice that received *Cbl-b*$^{-/-}$ or *C373A*$^{KI/KI}$ NK cells were found to have significantly fewer metastases than those that received wild-type NK cells.

In vitro ubiquitylation assays identified the TAM family (TYRO3, AXL and MER) of receptor tyrosine kinases as substrates of CBL-B. The activation of the TAM receptors by their endogenous ligand GAS6 (growth arrest-specific protein 6) led to the recruitment of CBL-B and ubiquitylation of AXL, which resulted in suppression of NK cell activity. *Cbl-b*$^{-/-}$ NK cells were resistant to the GAS6-mediated negative regulation of NK cells, indicating that CBL-B also acts downstream of GAS6 and the TAM receptors.

The authors then developed the highly selective small-molecule TAM kinase inhibitor LDC1267. *In vitro*, this inhibitor abolished the inhibitory effect of GAS6, and enhanced NK cell cytotoxicity towards cancer cells. *In vivo*, administered either intraperitoneally or orally, LDC1267 was well tolerated and reduced metastasis in mouse models of melanoma and breast cancer.

Interestingly, the anticoagulant warfarin is known to prevent the γ-carboxylation of TAM ligands, rendering GAS6 unable to activate TAM receptors. The authors therefore investigated whether the GAS6–TAM–CBL-B negative regulatory axis in NK cells might explain the decade-old conundrum of why warfarin has anti-metastatic activity. Indeed, the administration of low-dose warfarin, which inhibits TAM receptor activity without affecting coagulation, markedly reduced metastasis in a mouse model of melanoma in wild-type mice, but not in *Cbl-b*$^{-/-}$ mice. The depletion of NK cells in these models abolished the effect of warfarin on tumour metastasis.

In summary, these results show that CBL-B, via its E3 ligase domain, negatively regulates the anti-metastatic potential of NK cells, and provide a molecular explanation for the anti-metastatic activity of warfarin. Importantly, they indicate that it is possible to develop a 'pill' that awakens the innate immune system to kill cancer metastases.

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ORIGINAL RESEARCH PAPER Paolino, M. *et al.* The E3 ligase Cbl-b and TAM receptors regulate cancer metastasis via natural killer cells. *Nature* http://dx.doi.org/10.1038/nature12998 (2014)