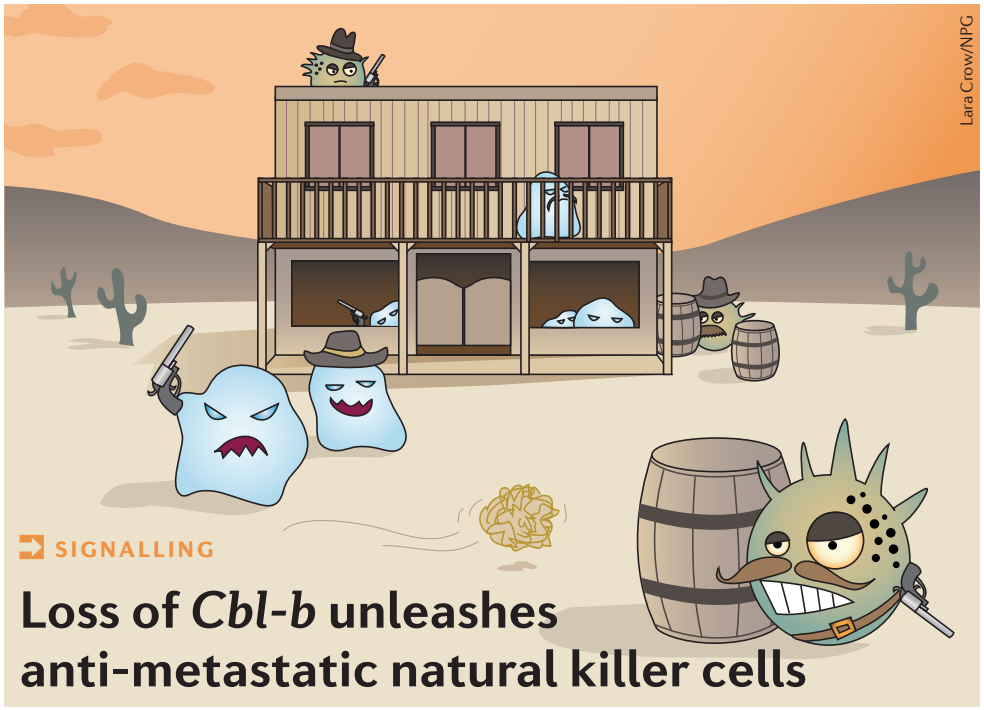
Nature Reviews Cancer | AOP, published online 13 March 2014; doi:10.1038/nrc3707



SIGNALLING

Loss of *Cbl-b* unleashes anti-metastatic natural killer cells

> deletion or inhibition of casitas B-lineage lymphoma-b (Cbl-b) decreases metastasis ... by activating natural killer (NK) cells

Despite several advances in cancer treatment, metastasis remains a major therapeutic challenge. A new study by Paolino *et al.* has shown that deletion or inhibition of casitas B-lineage lymphoma-b (*Cbl-b*) decreases metastasis in various mouse tumour models by activating natural killer (NK) cells.

Previous studies from this group and others established that deletion of *Cbl-b* or specific inactivation of its E3 ubiquitin ligase activity in mice caused rejection of multiple tumour types and that this was mediated by the activation of $CD8^+$ T cells. The authors sought to clarify these results and observed that xenograft tumour growth was significantly delayed in *Cbl-b*$^{-/-}$ mice and that this was concomitant with an increase in NK cells in tumours from these mice.

Both *Cbl-b*-deficient NK cells and NK cells that lacked CBL-B E3 ubiquitin ligase activity ($C373A^{KI/KI}$ cells) showed increased proliferation and increased interferon-γ (IFNγ) production when activated *in vitro*. In addition, both *Cbl-b*$^{-/-}$ mice and $C373A^{KI/KI}$ mice exhibited fewer metastases or smaller metastatic foci in several models of metastasis. This strongly indicates that CBL-B and, in particular, its E3 ubiquitin ligase activity are required for metastasis in mice.

In order to determine the substrates of CBL-B, the authors screened 9,000 human proteins, and they identified the tyrosine kinase receptor TYRO3, together with its TAM (TYRO3, AXL and MER) family members as substrates for CBL-B ubiquitylation.

As the TAM receptors are known to negatively regulate other immune cells, such as macrophages, the authors next determined whether they could suppress NK cells. Activation of TAMs in cell culture with their ligand GAS6 (growth arrest-specific protein 6) suppressed the growth and IFNγ production of NK cells, but NK cells that lacked *Cbl-b* did not show this effect.

The authors then developed an inhibitor (LDC1267) to the TAM tyrosine kinase receptors, in order to determine whether inhibition of TAMs could mimic the loss of *Cbl-b* and prevent metastasis in a mouse model. This inhibitor successfully decreased lung metastasis in a melanoma metastasis model, as well as liver metastasis in an orthotopic breast cancer model, although it did not have an effect on the primary mammary tumour. In addition, LDC1267 did not decrease anti-metastatic activity in *Cbl-b*$^{-/-}$ mice, which further supports the idea that TAMs function upstream of CBL-B.

Finally, the authors showed that the hitherto unknown mechanism that underlies the anti-metastatic activity of the coagulant warfarin is due to its inhibition of TAM receptors. Treatment with very low doses of warfarin (which do not affect coagulation) reduced melanoma metastasis but had no anti-metastatic effect in *Cbl-b*$^{-/-}$ mice, thereby mimicking the activity of the TAM inhibitors. This suggests that the anti-metastatic properties of warfarin function via the TAM and *Cbl-b* pathway.

This study offers insights into the activation of the immune system by inhibition of CBL-B and its ubiquitylation targets, the TAM receptors, to specifically target tumour metastasis. Furthermore, it implicates TAMs in the molecular mechanisms that underlie warfarin anti-metastatic activity, which may yield future therapeutic avenues. It remains unclear whether CBL-B affects NK activation solely through TAMs or through interaction with other pathways as well.

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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)