METASTASIS

# Deciphering signatures

What is the basis of the tissue tropism shown by metastatic cells? Joan Massagué and colleagues now report a gene-expression signature that distinguishes breast cancer cells that metastasize to bone from those that metastasize elsewhere.

Massagué and colleagues have previously shown that the human breast cancer cell line MDA-MB-231 has a poor-prognosis metastasis signature and have subsequently identified and validated another set of genes in this cell line that specifically mediates bone metastasis in the mouse. In this study, single-cell-derived progenies (SCPs) derived from MDA-MB-231 were introduced into the arterial circulation of immunodeficient mice. The development of bone metastases was followed by transducing the SCPs with a triple-modality reporter gene and tracking the metastatic cells with bioluminescence imaging and fluorescence microscopy. Bone was the main site of tumour growth, but the SCPs varied in the aggressiveness of their growth in bone and this correlated with whether or not they expressed the bone-metastasis gene set. A few SCPs grew in the adrenal glands and, if tail-vein injection was used, some SCPs grew in the lung. SCPs that grew well at one site did not necessarily grow well at other sites, consistent with the hypothesis that growth at metastatic sites is increased by genes that confer favourabletumour–stroma interactions at that site.

So are there differences in gene expression that can account for the variability in metastatic activity of the SCPs? The authors found 286 genes that differed more than twofold in their expression between SCPs. Classification of the gene-expression profiles showed that SCPs that had different primary metastatic tropisms — bone or lung — or were weakly metastatic formed distinct clusters. These three clusters were significantly closer to each other than to the profile of a normal human breast epithelial cell line. These data support the idea that distinct gene-expression patterns are responsible for variation in metastatic tropism.

ONCOGENES

# Addicted to MYC?

Studies in a range of model systems have indicated that tumours can become 'addicted' to the oncogenes that initiated them, so it might be possible to treat cancer by transiently targeting a single dominant pathway. For example, inactivation of *MYC* in experimental tumour systems has been shown to reverse the malignant properties of a range of tumour types. Lewis Chodosh and colleagues report, however, that this is not the case for mammary adenocarcinoma cells, making targeted therapy more of a challenge than expected.

Several transgenic mouse models have been used to show that even advanced-stage tumours can remain dependent on pathways activated by individual oncogenes such as *MYC* or *RAS*. So targeting a single one of these pathways would lead to regression of tumours, in spite of many other genetic and epigenetic alterations. These findings are at odds with clinical findings, however, as patients with metastatic cancers of epithelial tissues are rarely cured by a single agent or even by combination therapy. Chodosh and colleagues developed a transgenic mouse model of breast cancer that more closely resembles a human epithelial tumour type — as MYC amplification occurs in 5–15% of human breast cancers, they used an inducible promoter to overexpress this oncogene specifically in the mammary glands.

These mice develop mammary adenocarcinomas, but in contrast to other mouse models of MYC activation, less than half of these tumours regressed following *MYC* transgene downregulation — most of these tumours rapidly acquire the ability to grow in the absence of MYC overexpression. Furthermore, over half of the fully regressed tumours recurred spontaneously, without MYC overexpression, at the site of the original tumour. And the tumours that did not spontaneously recur did rapidly grow back when *MYC* was briefly re-expressed, indicating the presence of residual cells that are only one step away from re-acquiring their full malignant potential. So transient inactivation of this oncogene would not be sufficient to stop tumour progression of mammary epithelial cells.

What allows these tumours to survive in the absence of MYC? Chodosh's group showed that many *MYC*-independent mammary tumours harboured *KRAS* mutations, but multiple other mechanisms are likely to underlie *MYC* independence. The authors propose that in some types of cancer, such as haematological cancers or sarcomas, *MYC* inactivation leads to cell differentiation, which can prevent tumour recurrence. The fact that mammary tumour cells and other epithelial cell types do not undergo terminal differentiation following MYC downregulation might explain their resistance to *MYC* inactivation. So oncogene dependence seems to be context dependent, and common human epithelial cancers are likely to require chronic and combination treatments with targeted agents.

*Kristine Novak*

## References and links

**ORIGINAL RESEARCH PAPER** Boxer, R. B., Jang, J. W., Sintasath, L. & Chodosh, L. A. Lack of sustained regression of c-MYC-induced mammary adenocarcinomas following brief or prolonged MYC inactivation. *Cancer Cell* **6**, 577–586 (2004)

To investigate the relevance of these findings to the behaviour of disease in humans, 63 primary breast cancers were examined for expression of 50 genes from the bone-metastasis gene set that are also present in the poor-prognosis signature. Hierarchical clustering did not distinguish between tumours that had metastasized to bone and those that had not. However, when the analysis was restricted to those tumours that were known to have metastasized, the 50-bone-metastasis gene set did distinguish a bone-metastasis cluster from a lung-metastasis cluster.

Confirmatory studies could lead to an accurate predicter of bone-metastasis tropism in primary breast cancers, which would be valuable in the effective management of breast cancer patients.

*Ezzie Hutchinson*


**References and links**

**ORIGINAL RESEARCH PAPER** Minn, A. J. *et al.* Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. *J. Clin. Invest.* **115**, 44–55 (2005)

**WEB SITE**

**Joan Massagué's lab:** http://www.hhmi.org/research/investigators/massague.html


THERAPEUTICS

# On the TRAIL of death

**Histone-deacetylase inhibitors (HDACIs) have successfully entered clinical trials, but the basis of their antitumour activity is not clear. Two papers published in the January issue of *Nature Medicine* indicate that HDACIs increase the expression of the death-receptor ligand TRAIL in cancer cells, leading to tumour-cell death.**

Histone deacetylases (HDACs) regulate transcription by altering chromatin structure and can also modify individual protein function. Their activity is frequently altered in human tumours. The best-characterized example of this is evident in myeloid leukaemia cells, where the oncogenic, chromosomal translocation fusion protein products PML–RAR or AML1–ETO function to silence genes and transform cells by interacting with HDACs.

Insinga and colleagues treated mice with PML–RAR-induced acute promyelocytic leukaemia (APL) with the HDACI valproic acid and compared their response with standard therapy for APL, all-*trans* retinoic acid. Both drugs prolonged the survival of these mice, but through different mechanisms — all-*trans* retinoic acid primarily induced the terminal differentiation of the leukaemic blast cells, whereas valproic acid induced massive blast-cell apoptosis. The authors found that the pro-apoptotic activity of valproic acid is not due to the inhibition of the PML–RAR-induced degradation of the tumour-suppressor gene product p53, but that treatment with HDACIs induces the selective upregulation of the death receptors DR5 and FAS and their cognate ligands TRAIL and FASL. Blocking access of these ligands to their receptors through blocking antibodies prevented valproic-acid-triggered apoptosis *in vitro* and RNA-interference studies confirmed this result *in vivo*. These authors show further that the effect of HDACIs is reproduced in other mouse leukaemic models and in a subset of freshly isolated human leukaemic blasts.

Nebbioso and colleagues examined the action of three HDACIs — including the benzamide derivative MS275 — on a human leukaemic cell line and a large number of blasts from patients with acute myeloid leukaemia (AML). They found that TRAIL expression and resultant apoptosis were induced and, in addition, that MS275 induced cell-cycle arrest, upregulation of the cell-cycle inhibitor p21(WAF1) and differentiation of the leukaemic cells. To examine the contribution of p21 and TRAIL to HDACI antitumour activity, these authors used RNA interference to knockdown the expression of these proteins. Their results show that p21 specifically induces HDACI-mediated growth arrest and that TRAIL induces the acute apoptotic response through the death-receptor pathway. Moreover, irrespective of their genetic defects, most *ex vivo* cultured AML blasts from patients responded to HDACI exposure. Nebbioso and co-workers also showed that MS275 induces the expression of the TRAIL gene *TNFSF10* by inhibiting promoter-resident HDAC1 and HDAC2 and recruiting and acetylating the transcription factors SP1 and SP3, allowing formation of a transcriptionally active complex.

Significantly, both groups show that no apoptosis was evident in the normal myeloid progenitors tested, despite their intrinsically higher levels of TRAIL expression. Both conclude that the HDACI-mediated selective TRAIL expression and apoptosis seen in myeloid leukaemic cells warrants further investigation and has implications for the treatment of other human tumours.

*Nicola McCarthy*


**References and links**

**ORIGINAL RESEARCH PAPERS** Insinga, A. *et al.* Inhibitors of histone deacetylases induce tumor-selective apoptosis through activation of the death receptor pathway. *Nature Med.* **11**, 71–76 (2005) | Nebbioso, A. *et al.* Tumor-selective action of HDAC inhibitors involves TRAIL induction in acute myeloid leukemia cells. *Nature Med.* **11**, 77–84 (2005)