METASTASIS

A key to metastasis

As metastasis of primary cancers is the main cause of mortality in individuals with cancer, the identification of proteins that are important for this process is essential. Now, two groups report in *Nature Medicine* that ezrin is upregulated in metastatic cancers, and is key to the metastatic process.

Glenn Merlino and colleagues used a rhabdomyosarcoma mouse model to establish cell lines that were either highly or poorly metastatic when injected into mice. They then performed microarray expression profiling to identify genes that were differentially expressed. Two genes that were highly overexpressed — confirmed by western and northern blots — in the metastatic cell lines were those that encode ezrin and the homeoprotein transcription factor Six1.

But do they really contribute to metastasis? Overexpression of either gene in poorly

THERAPEUTICS

Unleashing p53

The transcription factor p53 controls a key pathway protecting cells from malignant transformation. In many cancers, however, this protective activity is switched off, commonly as a result of overexpression of the protein MDM2, which binds to the transactivation domain of p53 and blocks its ability to activate transcription. So, inhibiting the binding of MDM2 to p53 has been suggested as an anticancer



metastatic cell lines increased their metastatic potential in mice by several fold. Similarly, disruption of the genes in highly metastatic cell lines diminished their metastatic

ability. Interestingly, overexpression of Six1 seems to induce ezrin expression, forging a link between these two metastasis factors. Human rhabdomyosarcomas also have increased expression of these factors, which correlates with the disease stage.

Having previously identified ezrin in a genomic screen for metastasis-associated genes, Lee Helman and colleagues first confirmed its requirement by showing that its disruption — using an antisense approach or a phosphorylation mutant that keeps ezrin in the closed conformation — decreased metastasis in an experimental mouse osteosarcoma system.

They then used a single-cell fluorescentimaging approach to follow the fate of cells that arrive in the lungs during metastasis. Cells in which ezrin was suppressed soon underwent apoptosis, so might ezrin inhibit anoikis? This would allow cells to survive in the lungs until they can attach to the tissue. The Akt and mitogen-activated protein

strategy, a proposal that is given support by a recent paper in *Science* describing small-molecule antagonists of the MDM2–p53 interaction that activate the p53 pathway in cancer cells and inhibit tumour growth in mice.

Protein-protein interactions have traditionally been viewed as highly challenging targets for small-molecule drug discovery, owing to issues such as the lack of well-defined binding pockets at the interface. However, the crystal structure of MDM2 bound to a peptide from the transactivation domain of p53 revealed that MDM2 possesses a relatively deep pocket that is filled by a helical region of the p53 peptide, raising the hope of identifying small molecules capable of binding in the pocket instead of p53. Vassilev and colleagues therefore screened a diverse library of compounds for their ability to inhibit the binding of MDM2 to p53 using surface plasmon resonance, which is well-suited to analysing protein-protein interactions, and discovered a series of cis-imidazoline analogues that displace p53 from its complex with MDM2 with IC₅₀s in the nanomolar range. A crystal structure of one of the compounds in complex with MDM2 confirmed that it bound in the p53-binding site.

Testing the imidazoline analogues in a range of cancer-cell-based assays provided strong evidence that they activated the p53 pathway, leading to cell-cycle arrest and apoptosis. kinase (MAPK) pathways have been implicated in anoikis inhibition, and ezrin was shown to decrease their activity. A constitutively active component of the MAPK pathway (Mek), but not activated Akt, was able to partially reconstitute metastasis in ezrin-suppressed cells.

Finally, the authors investigated the expression of ezrin in the highly metastatic spontaneous osteosarcomas that occur in pet dogs, as they are more relevant to humans. Ezrin was overexpressed in 83% of primary tumours, and its expression intensity correlated with the more aggressive tumours. Preliminary analysis indicates that the same is true in human paediatric osteosarcomas, which indicates that ezrin is an important regulator of metastasis that should be investigated further.

Emma Greenwood

References and links

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FURTHER READING McClatchey, A. I. Merlin and ERM proteins: unappreciated roles in cancer development? *Nature Rev. Cancer* **3**, 877–883 (2003)

Encouraged by this, the authors assessed whether one of the analogues could suppress the growth of established tumour xenografts in mice. Oral administration of the compound, which was well-tolerated, resulted in 90% inhibition of tumour growth relative to vehicle controls, compared with 81% inhibition using intravenous administration of the maximal tolerated dose of the traditional cytotoxic drug doxorubicin.

Although ~50% of human tumours have lost wild-type p53 and so would not be expected to be affected by inhibitors of the p53–MDM2 interaction, the authors' experiments indicate that activating the tumour-suppressor capability of p53 with such compounds might be beneficial in the other ~50% of cancers in which the wildtype form of p53 is retained. More generally, their demonstration that a protein–protein interaction can be successfully targeted by small-molecule inhibitors provides encouragement for the growing number of research programmes pursuing this challenging goal.

Peter Kirkpatrick

Senior Editor, Nature Reviews Drug Discovery

Beferences and links

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