EARLY DETECTION

Tiny signals

In patients with breast cancer, biopsies for lymph node metastases are usually performed to determine the patient prognosis — those who test positive are considered as candidates for adjuvant therapy. However, up to 30% of patients that are free of lymph node metastases still develop metastatic disease. In the *New England Journal of Medicine*, Stephan Braun *et al*. report that the detection of disseminated tumour cells in bone marrow samples is a more reliable determinant of metastasis and patient survival.

Unlike other tumour types, such as head and neck cancers, breast tumour cells frequently bypass the lymph nodes and disseminate directly through the blood to distant organs. This haematogeneous dissemination of cancer cells has been shown to be an early event in tumour progression. Small numbers of disseminated tumour cells can be detected in bone marrow samples by sensitive immunocytochemical assays for proteins such as cytokeratin and epithelial mucins, so Braun *et al*. set out to determine their prognostic significance. In a pooled meta-analysis of data from 9 studies involving 4,703 patients with stage I, II or III breast cancer, the authors evaluated the association between detection of bone marrow micrometastases and patient outcome over a 10-year period.

The study compared the effects of different factors, such as tumour size, grade, bone marrow metastasis, lymph node metastasis and hormone receptor expression, and revealed that the presence of micrometases in bone marrow were the best predictors of outcome (survival, disease recurrence and metastasis to other organs) within the first 5 years after diagnosis. Bone marrow micrometastases were detected in over 30% of patients and these patients were found to develop larger tumours with a higher histological grade that more

STRUCTURE

InhibitABL

Although the kinase activity of BCR–ABL is inhibited by imatinib, the onset of drug resistance has prompted the search for further intervention strategies. Aside from its increased tyrosine kinase activity, the primarily cytoplasmic localization of the fusion protein is thought to contribute to its leukaemogenic properties. Because the F-actin binding domain (FABD) in the carboxy terminus of BCR–ABL is thought to, in part, regulate this localization, Oliver Hantschel and colleagues have analysed its three-dimensional structure.

ABL localizes to both the nucleus and the cytoplasm — this is dependent on environmental cues. However, BCR–ABL does not, but both proteins have identical carboxy terminal domains that govern the localization of the protein. These include three nuclear localization signals that enable the import of the kinase into the nucleus, a putative nuclear export signal that is part of the FABD, and the FABD itself. The authors determined the 3D structure of the FABD of human ABL by heteronuclear nuclear magnetic resonance spectroscopy. They found that the FABD folds into a compact bundle of four antiparallel α-helices and the 3D structure of this domain shows strong homology to other cytoskeletal proteins, some of which also bind F-actin. In contrast to previous observations, the authors show that the nuclear export signal is non-functional and is part of the hydrophobic core of the FABD, unless artificially exposed as an isolated peptide.

How does the FABD affect the localization of ABL and BCR–ABL? To address this, the authors made 21 mutant ABL and BCR–ABL proteins based on the structure of the FABD and examined their localization in cultured cells and their interaction with purified F-actin in a cell-free system. Their results indicate that loss of the FABD in ABL causes nuclear localization, but this does not occur in BCR–ABL. As both FABD are the same, there must be another property of the fusion protein that keeps it tethered in the cytoplasm. The authors' preliminary evidence indicates that the increased kinase activity of the fusion protein is not sufficient to exclude BCR–ABL from the nucleus, but that the coiled-coil domain of BCR is important.

Irrespective of the precise mechanism, previous data have indicated that disrupting the FABD domain of BCR–ABL limits its oncogenicity. The authors mapped the F-actin binding site to a few conserved residues in an α-helix. Based on this and their structural data, the authors conclude that pharmacological disruption of these conserved residues is a plausible approach for inhibiting BCR–ABL.

Nicola McCarthy

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FURTHER READING Ruibao Ren. Mechanisms of BCR–ABL in the pathogenesis of chronic myelogenous leukaemia. *Nature Rev. Cancer* **5**, 172–183 (2005)

WEB SITE

Giulio Superti-Furga's lab: http://www.cemm.oeaw.ac.at/?cont=people&sub=gsf

frequently metastasized to other organs. Furthermore, patients with bone marrow micrometastases were more likely to die of the disease, even among patients that received adjuvant therapy. In patients with very small tumours (less than 2 cm in diameter) without lymph node metastases, and who therefore did not receive adjuvant therapy, the presence of bone marrow micrometastases was also associated with shorter survival times.

The authors suggest that assays for the presence of bone marrow micrometastases could be a complementary approach to lymph node biopsies in determining which patients should receive adjuvant therapy.

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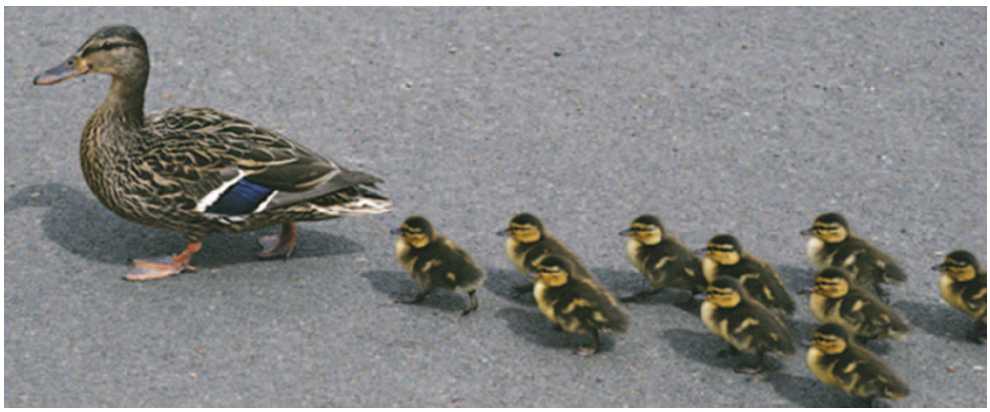
TUMOUR SUPPRESSORS

New additions



The genes that encode the p53 family members p63 and p73 enable the production of several different protein isoforms. In light of this fact, Jean-Christophe Bourdon, David Lane and colleagues have re-investigated the gene structure of the founding member, *TP53*. They found that *TP53* in fact encodes at least six different p53 mRNA isoforms, some of which are differentially regulated in cancer cells.

Mammalian genomes contain three members of the *TP53* family, yet only one form exists in invertebrates, implying that the mammalian members are derived from the triplication of one ancestral gene. If this hypothesis is correct, it is somewhat strange that *TP53* does not share the complexity of *TP63* and *TP73*, both of which can be transcribed from an alternative internal promoter and express at least 3 and 11 alternatively spliced isoforms, respectively. *TP53*, on the other hand, was thought to have a much simpler structure with only one promoter that transcribes three mRNA splice variants.

To assess *TP53* and all of its encoded mRNAs, Bourdon *et al.* used GeneRacer PCR, a technique that amplifies only capped mRNA transcripts and so allows the detection of the transcription initiation site. They also designed specific primers for exons four and five in an attempt to identify any transcripts that might be generated from an internal promoter.

The authors found that, altogether, *TP53* can theoretically transcribe nine different p53 isoforms. These are full length p53, p53β and p53γ, Δ133p53, Δ133p53β and Δ133p53γ (Δ133 owing to alternative internal promoters in intron four), and Δ40p53, Δ40p53β and Δ40p53γ (Δ40 owing to the alternative splicing of intron two or use of an alternative translation-initiation site). The β and γ isoforms arise from alternative splicing of intron nine. All of these mRNAs can be detected in normal human tissue samples in a tissue-specific manner and all of these mRNAs lead to protein expression. Endogenous p53β isoforms were detected by specific antibodies. However, isoform-specific antibodies still need to be generated to detect endogenous p53γ and Δ133p53 protein isoforms.

Do any of these isoforms affect p53 function? Further investigations showed that the p53β isoform binds the *BAX* promoter more readily than the *MDM2* promoter (or the *CDKN1A* promoter), whereas p53 preferentially binds *MDM2* over *BAX*. The authors show that this causes p53β to enhance p53-mediated *BAX* promoter activity, but that this does not seem to affect the level of apoptosis induced in cells expressing both p53 and p53β. On the other hand, Δ133p53 inhibits p53-mediated apoptosis, indicating that it can function as a dominant negative.

The authors also assessed expression of the mRNA isoforms in human breast tumour samples. None of the 30 samples expressed the same combination of p53 isoforms that is seen in normal breast tissue. For example, *TP53γ* and *TP53β*, which are expressed in normal breast tissue, were either not detected or detected in only ten samples, respectively. *Δ133TP53*, which is not expressed in normal breast tissue, was detected in 24 samples. Notably, only five of the tumours expressed a mutant form of p53.

On the basis of these findings, the authors conclude that the regulation of expression of the p53 isoforms seems to be altered in breast cancer, a finding that could be relevant in tumours that express wild-type p53.

Nicola McCarthy

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