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Stopping the spread of cancer

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Can blocking a single enzyme be enough to stop the spread of cancer? Scientists at Copenhagen University have recently published research showing this might be possible. They have observed that the spread of cancer can be prevented in mice missing a specific enzyme – urokinase plasminogen activator (uPA). The work, published in the *International Journal of Cancer*, shows that cancer doesn't advance unless uPA is present [1] (Figure 1). An additional boon to the development of cancer therapy is that the body does not appear to need uPA for essential functions.

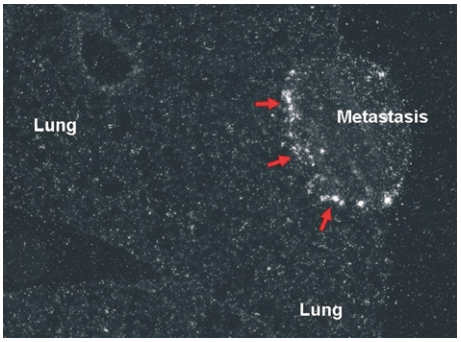

FIGURE 1

The urokinase plasminogen activator (uPA) is expressed at the invasive front of a lung metastasis from a transgene-induced breast cancer model. Dark field image of radiolabeled *in situ* uPA mRNA hybridization. Photograph kindly supplied by K. Almholt.

Hijack

uPA plays a number of physiological roles. It is perhaps best known for its role in wound healing. It is generated in healing wounds by skin cells but only at the very tip of their migration across the wound. 'It's this very focussed ability that's effectively being hijacked by the cancer cells,' said Almholt. Cancer cells don't make uPA, but they can instruct other cells to make uPA. The mechanism through which the cancer exerts this control is not yet known.

uPA catalyses the local conversion or activation of plasminogen to plasmin. But uPA is only one of the mediators that can perform this action. Tissue-type plasminogen activator (tPA) is the other main activator of plasminogen. Although plasminogen activators effectively perform the same function, only uPA has been shown to have a role in the development of cancers. There is also the potential for the different members of the family 'substituting' for one another. In wound healing, for example, tPA can take on the functions of uPA in its absence. This is where Almholt and his colleagues can see advantages for drug development at uPA. 'This is where we see the true beauty of this system,' said Almholt. If uPA – and all its cancer-related actions – is 'taken out,' its physiological functions can be taken over by tPA, thus minimizing any side effects. 'Evidence for this comes from studies on mice with double knockouts – minus tPA and uPA,' said Almholt. 'These mice do have problems with wound healing.'

A bigger player

The field of uPA research in general is huge – and work in the area has been ongoing for some years now. 'Researchers have been systematically working through their favourite challenges. This research is somewhat exciting, but just the next step down this path' said Gillian Murphy, professor of cancer biology at Cambridge University, and funded by Cancer Research UK. 'uPA is important,' Murphy continued. 'It's now clear that uPA is a bigger player in cell biology.' Murphy believes this research is 'good further evidence' supporting a strong hypothesis implicating uPA in the spread of cancer. 'It is interesting that uPA doesn't prevent cancers developing, but the metastases are halted,' continued Murphy. Other groups are also exploring this quirk that, apparently applies to other systems too. A recent paper in the *Journal of Cell Biology* by researchers at the Scripps Research Institute focussed on preventing the metastases of cancers by inhibiting another enzyme – Src kinase – rather than targeting the cancer cells themselves [2].

New drugs

How, then, could this work lead to the development of new drugs? 'There are urokinase inhibitors out there, but they're not specific,' added Almholt. 'There are some trials currently ongoing, but they're not truly specific as they also act on other serine proteases.' Blocking the conversion of plasminogen to plasmin completely can prove significant. Being a ubiquitous protein, plasmin has a physiological role throughout the body – inhibiting it would mean serious side effects. 'This has been observed in plasmin deficient mice,' continued Almholt. 'They are indeed very ill.'

One of the agents currently in development is Wilex's uPA inhibitor WX-UK1,

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currently in Phase I/II trials. The German-based company have developed a small molecule that inhibits uPA, together with other serine proteases. Wilex are continuing their research with the WX-678 series of potent and selective small molecule uPA inhibitors, currently at the preclinical stage of development.

Although not actively involved in drug discovery, Almholt and colleagues are looking to the future. 'What we'd like to do is find a specific uPA inhibitor and test in same model,' says Almholt. 'This might be a small molecule, or more likely we'll look to antibodies specific to the mouse uPA.'

References

1 Almholt, K. *et al.* (2004) Reduced metastasis of transgenic mammary cancer in urokinase-deficient mice. *Int. J. Cancer* 113, 525–532

2 Weis, S. *et al.* (2004) Endothelial barrier disruption by VEGF-mediated Src activity potentiates tumor cell extravasation and metastasis. *J. Cell Biol.* 167, 223–229

Wide-angle X-ray scattering for screening functional ligands

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'Seeing' functional protein–ligand interactions is now possible through the novel application of an established biophysical technique. Researchers at the Argonne National Laboratory (IL, USA) have just demonstrated that ligand binding that induces any type of conformational change in the secondary, tertiary or quaternary structure of the protein can be detected using wide-angle X-ray scattering (WAXS).

Multiple length scale detection

Spotting when small molecules bind functionally to proteins to screen for potentially useful drug candidates has proved difficult. Nuclear magnetic resonance spectroscopy, small-angle X-ray scattering and X-ray crystallography can observe some of the conformational changes that occur, but each sees its own limited view. 'In addition, some of techniques, notably X-ray crystallography, require good crystals, significant amounts of protein and are laborious, making it difficult to do in a moderate-throughput fashion,' says lead author Robert Fischetti. WAXS detects structural change across multiple length scales – 'in other words, it can detect changes in structure at a scale from changes in position of individual amino acid residues in the active site all the way to hinge rotations of entire protein domains,' he explains. This is unique among the biophysical approaches to observation of conformational changes.

Tom Gadek (Chief Scientific Officer, SARcode, Oakley, California, USA) comments that he is 'very impressed by the sensitivity of the technique, particularly in the case of adipocyte lipid-binding protein'. In this protein, he observes, the binding of a lipid molecule can be easily detected even though it has no effect on the backbone alpha carbon fold of the protein and the reported WAXS difference signal arises from a reorientation of just three surface residue sidechains. Glenn Hammonds (Principal Scientist, Information Biology Consulting, Berkeley, California, USA) agrees that 'the ability of WAXS to rapidly detect ligand binding seems well demonstrated.' He adds that 'it could be useful in the absence of a specific and sensitive assay system, or if there is a need to distinguish among a limited number of ligands on the basis of their effect on conformation.'

Clearing a bottleneck in drug discovery

However, he cautions that the potential of the method to clear a bottleneck in pharmaceutical drug discovery depends on three factors. Firstly, whether most proteins do undergo some degree of conformational change on binding to a ligand, as the authors believe, secondly, whether detection of low affinity (5 mm Kd) hits is a desirable characteristic of an initial screen and, finally, on the economics of protein production. 'These requirements present some difficulties, since it is not at all clear what proportion of ligand binding events will produce a clear conformational signal; most traditional screening assays are geared toward detecting somewhat stronger interactions,' he says.

Fischetti and colleagues are convinced that the technique has proved itself well enough to identify small molecule ligands that alter the function of a protein that is central to a specific disease process. 'We are presently looking for collaborators to work with on an ongoing angiogenesis project at Argonne,' he reports. The group also looks forward to increasing the speed of the technique and scaling it up. 'This study was successful because of third-generation synchrotron sources. To really go to high throughput, one would need a dedicated facility, but given the speed and breadth of the technique, a single facility could serve a large number of projects,' concludes Fischetti.

Novel approach to combating superbugs

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With superbugs now ubiquitous in many health care settings, treating them becomes increasingly difficult as therapeutic options dwindle. Devising new strategies to overcome resistance has never been more crucial. Taking a novel approach to combating superbugs, biochemist Paul Hergenrother and colleagues at the University of Illinois, Urbana, IL, have devised a method to overcome resistance by targeting the DNA that renders them antibiotic-resistant.

The resistance problem

In recent years, only one new class of antibiotics with a novel mechanism of action surfaced from the pharmaceutical pipeline since

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