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Cleaning up the environment

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One vexing problem with cancer is that even if the disease appears to be in remission it often comes back. Although it has long been suspected that cancerous cells were modifying their environment, this was not well understood until 2001 [1]. Mary Hendrix, Department Chair of Anatomy and Cell Biology at the University of Iowa (http://www.uiowa.edu), discovered that aggressive melanoma cells overexpress certain proteins, which modify surrounding cells. Her group has followed up this paper with another, which shows that COL-3, a chemically modified tetracycline (CMT), inhibits both expression of these proteins and their action on surrounding cells.

Creating a favourable habitat for cancer

Aggressive melanoma cells overexpress several proteins, including lamin 5 (Ln5) γ2 chain, which is overexpressed 50-fold in aggressive cells but not produced by non-aggressive cells. The aggressive cells also overexpress metalloproteinases (MMPs), such as MMP2 and membrane type 1 (MT1)-MMP. The proteins then act co-operatively to change the tumour environment by cleaving the γ-chain. The fragments are then incorporated into the extracellular matrix.

The evidence shows that these fragments can induce aggressive behavior in surrounding cells, causing them to engage in vasculogenic mimicry when cultured on a 3D matrix [1]. Vasculogenic mimicry refers to the ability to express endothelial and vascular associated genes including many MMPs and vascular endothelial (VE)-cadherin. The Hendrix group discovered this effect by studying non-aggressive MUM-2C melanoma cells, which were grown on a 3D collagen matrix that had first been used by aggressive MUM-2B cells. The 3D matrix helps to replicate an environment that is more similar to an organ than a petri dish, thus enabling the cells to grow and interact more naturally.

'We put in the aggressive cells first and they leave behind a microenvironment,' explains Hendrix. 'Then we remove the aggressive cells and put in the non-aggressive cells and you see induction.'

Balakrishna L. Lokeshwar, Associate Professor of Urology at the University of Miami (http://www.miami.edu), explains that the microenvironment probably ensures that removing the tumour only causes other cells to grow in its place. 'This concept of the tumour microenvironment is similar to a plant making it's own fertilizer,' he says. 'Once you remove the plant, you leave behind the fertilizer and the other surrounding plants now start growing faster.'

Targeting the microenvironment

To inhibit microenvironment signaling, Hendrix chose COL-3, a chemically modified tetracycline that has antimetastatic [2,3] and antiangiogenic activity, and also inhibits MMP activity [4].

In the experiment, collagen plates were conditioned by aggressive MUM-2B cells, which were removed after four days. During this time, the cells overexpress Ln5 γ-2, which is cleaved by the MMPs. The γ-chain fragments then incorporate themselves into the collagen. Following removal of the MUM-2B cells, non-aggressive MUM-2C cells are seeded onto the plate and begin to grow in a network pattern that indicates an aggressive phenotype. The MUM-2C – which does not normally produce Ln5 γ-2 chains – also began producing the protein.

However, when MUM-2B cells were grown with COL-3, the cells changed and no longer grew in a network pattern. With PCR analysis, it was also found that they were inhibited from producing many vasculogenic markers, including MMP2, MMP9 and cadherin. Further, when the collagen matrix was tested, it was found to no longer include γ-2 signaling fragments, and when seeded with MUM-2C, the non-aggressive cells did not grow in a network pattern, nor did they produce Ln5 γ-2.

'The study shows that not only does COL-3 inhibit expression of vasculogenic markers, but it also inhibits cleavage of Ln5 γ-2,' says Hendrix. 'That means there is an inhibition of these signals which aggressive cells leave behind in the environment.'

Implications for future therapies

Describing the study as 'extremely elegant,' Lokeshwar says microenvironment work holds the possibility of combining new drugs with standard chemotherapy to wipe out these signals. 'The microenvironment is a hot new field because we've seen the failure of standard chemotherapy, especially with high density tumours.'

What is not well understood is the mechanism for COL-3 activity. As the Hendrix paper shows, the drug inhibits protein synthesis, but it's ability to block MMP enzymatic action is thought to be caused by chelating divalent cations. Both MMPs and COL-3 chelate these cations, with COL-3 being the better binder.

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Although there are numerous other chemically modified tetracyclines to test, COL-3 is the only one that is now in human trials. In Phase I clinical trials, the drug caused patients to become photosensitive. However, in the Phase II trial at the National Cancer Institute (http://www.nci.nih.gov), it was found that lowering the daily dose below 300 mg alleviated photosensitivity.

Hendrix says this paper only contained a small portion of her findings but is helping to build on two more studies. Working in collaboration with Paul Meltzer and Jeff Trent (as part of the Human Genome Project), Hendrix and colleagues have a manuscript in preparation that show the results of a 15,000 gene chip to find out which genes are being expressed as cells change and become aggressive.

She is also focusing on drug combinations that might work best with COL-3. 'In this paper, we suggest that COL-3 can be used in a combinatory manner, with COL-3 targeting messages or signals in the microenvironment and other drugs focusing on the tumour,' she says. 'So we are looking at the drug combinations which will give us this best outcome.'

References

1 Seftor, R.E.B. *et al.* (2001) Cooperative interactions of laminin 5 γ2 chain, matrix metalloproteinase-2, membrane type-1 metalloproteinase are required for mimicry of embryonic vasculogenisis by aggressive melanoma. *Cancer Res.* 61, 6322–6327

2 Seftor, R.E.B. *et al.* (1998) Chemically modified tetracyclines inhibit melanoma cell invasion and metastasis. *Clin. Exp. Metastasis* 16, 215–225

3 Lokeshwar, B.L. *et al.* (2002) Inhibition of cell proliferation, invasion, tumour growth and metastasis by an oral non-microbial tetracycline analog (COL-3) in a metastic cancer model. *Int. J. Cancer* 98, 297–309

4 Lee, H.M. *et al.* (2001) CMT-3, a non-antimicrobial tetracycline inhibits MT-1-MMP activity: relevance to cancer. *Curr Med. Chem.* 8, 257–260

News in brief

Targets and mechanisms

True mechanism of calcium channel blockers discovered

Researchers in America have hailed as 'a leap forward' their discovery of the true mechanism of action of calcium channel blockers [1]. The scientists, led by physiologist Mordecai P. Blaustein of the University of Maryland School of Medicine (http://medschool.umaryland.edu/), have demonstrated that the targets of the drugs are store-operated channels (SOCs), rather than voltage-gated channels as was previously believed.

Calcium has an important role in various functions in the body and alterations are thought to be responsible for disorders such as hypertension and angina. Not only do the new findings provide a real understanding of the function of calcium channel blockers, which are often prescribed to sufferers of these conditions, but they also lead the way for the development of novel drugs targeted specifically to SOCs, with fewer side-effects than therapies currently in use, explained Blaustein.

Using high-powered imaging on rat mesenteric arteries, the group made the discovery that both magnesium, a known blocker of SOCs, and nifedipine, a calcium channel blocker, abolished calcium entry through SOCs.

'Before this work, nobody recognized that [SOCs] were the main target of calcium channel blockers in increasing blood flow and lowering blood pressure,' said Blaustein. 'It may be possible,' he said, 'to identify new anti-hypertensive and anti-angina medications that target only [SOCs].'

1 Zhang, J. *et al.* (2002) Mg^{2+} blocks myogenic tone but not K^{+}-induced constriction: role for SOCs in small arteries. *Am. J. Physiol.* 10.1152/ajpheart.00260.2002 (http://ajpheart.physiology.org/)

Structure of LDL receptor extracellular domain revealed

Days could be numbered for the genetic disease familial hypercholesterolemia (FH) as a powerhouse team of scientists, including three Nobel laureates, revealed the 3D structure of the low-density lipoprotein (LDL) receptor extracellular domain [2].

FH, a disorder characterized by high cholesterol, atherosclerosis and an increased risk of heart attack is one of the most common 'single-gene' inherited diseases and affects around one in every 500 people, according to lead author of the study Gabrielle Rudenko. FH has previously been linked to about 1000 LDL receptor mutations and the recent identification of the receptor's structure will make it easier for scientists to understand how the disorder works, commented Rudenko. 'If you understand the basic biological mechanisms underlying a disease, you can hope to come up with strategies to battle [it],' she explained. 'In some cases, protein structures can even be used to design drugs.'

The team also included University of Texas Southwestern Medical Center (http://www3.utsouthwestern.edu/) researchers Johann Deisenhofer, Michael Brown and Joseph L. Goldstein, who have all won Nobel awards for their contributions to science. Deisenhofer, whose previous work has included the pioneering use of X-ray crystallography to show the structure of cell membrane proteins, said that the latest research would help scientists understand the mechanics of how our bodies absorb cholesterol from the blood.

2 Rudenko, G. *et al.* (2002) Structure of the LDL Receptor Extracellular Domain at Endosomal pH. *Science* 298, 2353–2358

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