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Smart bombs: the next generation of PDT

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Traditional chemotherapy works in much the same way as carpet bombing, destroying not only the cancer but also other cells throughout the body. Three researchers at Virginia Tech (http://www.vt.edu) are currently designing a smart bomb that directly targets cancer cells.

This targeting system not only provides more effective drug therapy but also reduces collateral damage to surrounding tissue.

Photodynamic therapy

Traditional photodynamic therapy (PDT) [1] selects a photochemical that preferentially accumulates in tumour cells. Once on target, a laser excites the chemical, which then generates oxygen radicals through electron transfer. By concentrating the photochemical and activating it only at the tumour site, two levels of protection are provided to surrounding tissue.

Designing the targeting system

The targeting system at Virginia Tech is being designed by Brian Storrie, Professor of Molecular Biology. With recombinant technology, he has expressed the B-subunit [2] of shiga toxin, which binds preferentially to a Gb3 (globotriaosyl ceramide) glycolipid. This glycolipid is overexpressed in certain types of malignancies, such as ovarian cancer.

Once the B-subunit has bound to the glycolipid, it is internalized and sequestered in the Golgi apparatus. 'This means the drug never enters the cytoplasm where it might interact with other molecules and cause toxicity,' says Storrie. 'It's a two-pass system – two levels of safety. You aim preferentially for the cancer cells, and then sequester it in the Golgi where you minimize side effects until you photoactivate.'

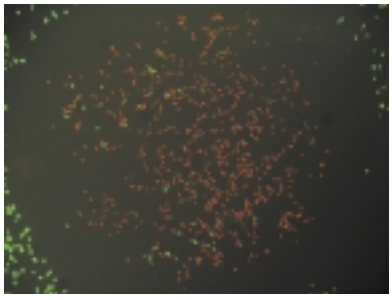

Figure 1. The figure shows viro cells (African Green Monkey kidney cells) that have endocytosed a two-part molecule consisting of a B-subunit of shiga toxin coupled to a porphyrin-like photosensitizer. The area in the centre has been illuminated, causing the photosensitizer to generate oxygen radicals. The red cells are dead (coloured by ethidium bromide) and cells outside the field of illumination are unharmed (green). Figure amplification ~10×. Figure courtesy of Brian Storrie, Professor of Molecular Biology at Virginia Tech (http://www.vt.edu)

Building a better bomb

Attached to the B-subunit is the bomb – a tri-metallic supramolecule created by Karen Brewer, Associate Professor of Chemistry at Virginia Tech. This molecule [3] consists of three subunits – two photosensitizers that absorb light and a bioactive site that kills the cell. Each of these components can be switched out, enabling the user to substitute different components to maximize efficacy.

As a laser pulses on the tumour, the osmium and ruthenium photosensitizers become excited and pass an electron to the bioactive site, rhodium. Having two different light absorbers maximizes the effective range of light wavelengths, which can be captured. The electron from the photosensitizers excites the rhodium bioactive site, creating a charged radical, which wreaks havoc in the Golgi apparatus, effectively killing the cell.

Using rhodium metal also adds a level of efficiency over traditional PDT, which creates oxygen radicals. 'Since we have a metal, we don't use an oxygen-dependent pathway,' Brewer says. 'One of the problems with oxygen is that it can be depleted in the environment so you end up not killing the cells. Our system doesn't have that problem.'

Initiating the blast

Ken Meissner, a Senior Researcher of Optical Science and Engineering at Virginia Tech., is in charge of triggering the bioactive rhodium. It is a challenging task because light does not easily pass through tissue. 'In the body there's a therapeutic window running from 650–950 nanometers,' says Meissner. 'This is the range where light propagates well; it's still scattered but not absorbed.' Below this range, organic materials (such as haemoglobin) absorb light, and above 950 nm water interferes.

'Light can absorb many centimeters into tissue, but it starts to balloon out [scatter and lose focus],' says Meissner. 'And you need to get enough light on target to activate the light absorbers.' (See Ref. 4.) He adds that Brewers molecule uses two photochemicals, which should make this process easier.

From theory to therapy

So far, the team has shown that Storrie's targeting system preferentially binds to malignant cells. By coupling his B-subunit to a traditional PDT molecule, he has also shown that the system kills the cancer without harming surrounding cells (Fig. 1). The next step

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is to couple the B-subunit to Brewer's metallic supramolecule.

'This is the next stage and it should take us a few months to work out the chemistry,' says Brewer. The process, she says, will involve adding an amine or carboxylic acid to her molecule and then coupling it to the B-subunit. 'We are also going to see how this supramolecule works alone,' she says. The molecule could also be used as an antimicrobial.

After testing the system on cells they then plan animal studies to optimize different laser parameters. Using multiple light wavelengths and pulsing techniques, Meissner says he will then work out how to best concentrate light on malignant cells.

'There are many different parameters, such as molecule concentration, efficiency of the photochemicals and the amount of light you can concentrate on the tumour,' Meissner adds.

References

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4 Jacques, S.L. and Wang, L-H. (1995) Monte Carlo modeling of light transport in tissues. In *Optical Thermal Response of Laser Irradiated Tissue*, (Welsh, A.J. and van Gemert, M.J.C., eds), pp. 73–100, Plenum Press

News in brief

Targets and mechanisms

Schizophrenia and bipolar disorder linked by a DRIP

New insight into the etiology of schizophrenia and bipolar disorder has been provided by a recent study looking at the levels of neuronal calcium sensor-1 (NCS-1) in the prefrontal cortex of patients with these conditions [1]. Abnormalities in the dopamine system have been postulated for both schizophrenia and bipolar disorder, although the exact sites of alterations remain elusive. However, the recent discovery of multiple receptor-interacting proteins (DRIPs) and their role in modifying and expanding the functionality of these receptors, has led to the suggestion that there could be altered levels of certain DRIPs in specific areas of schizophrenic and bipolar brains.

NCS-1 is present in neuronal cells throughout the brain and is a member of the DRIP family. It is present in high levels in the human prefrontal cortex and can form complexes with G-protein-coupled receptor kinase-2 and D2 dopamine receptor, making it a suspect molecule in schizophrenic and bipolar patients. In this study, 50% higher levels of NCS-1 were found in schizophrenic and bipolar patients than in controls and in patients with depression.

These findings support the hypothesis that abnormalities in dopamine receptor-interacting proteins (e.g. DRIPs), and now altered levels of NCS-1, could be associated with schizophrenia and bipolar disorder. Although schizophrenia and bipolar disorder might be induced by different factors, they could share the same mechanisms that cause the observed abnormal brain function. This study, therefore, has important implications for the treatment of these conditions.

1 Ok Koh, P. *et al.* (2003) Up-regulation of neuronal calcium sensore-1 (NCS-1) in the prefrontal cortex of schizophrenic and bipolar patients. *Proc. Natl Acad. Sci. U. S. A.* 100, 313–317

The proteasome: it's just so degrading!

The process of degrading proteins no longer needed by cells is essential in the normal growth, development and regulation of cells. Scientists at UT Southwestern Medical Center at Dallas (http://www3.utsouthwestern.edu/) have identified a new and surprising mechanism by which a class of enzymes responsible for the breakdown of proteins operates [2]. These findings have implications for understanding diseases such as Parkinson's and several forms of cancer.

Many diseases involve the inappropriate accumulation of unneeded or damaged proteins, and cells normally use an enzyme called the proteasome to remove these proteins by cutting them into small pieces. The proteasome, which is present in all higher cells, contains its active sites inside a cylinder-like shape with a gate that prevents the entry of normal cellular proteins, thereby protecting them from destruction. For years, scientists believed that the proteasome only degraded proteins tagged by a 'death marker' named polyubiquitin, which directed damaged proteins to a complex that opened the gate. It was thought that substrates accessed the internal catalytic sites of the proteasome by processively threading their termini through the gated substrate channel. However, new findings reveal that some important substrates do not need to be marked with polyubiquitin, but can open the gate themselves, enter the active cylinder and be degraded.

The scientists conducted the research by performing biochemical assays using a purified protein involved in Parkinson's disease and a cell-cycle regulator important for the progression of cancer. They found that the proteasome could independently degrade these proteins, cutting in the middle of substrates at internal peptide bonds in an endoproteolytic process. These findings may have implications for the development of future drugs to treat these diseases.

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