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Zebularine: a candidate for epigenetic cancer therapy

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Prospects look good for the development of zebularine, a stable DNA cytosine methylation inhibitor, as an epigenetic therapy for cancer with the report in *Cancer Cell* [1] by Peter Jones, Director of the USC/Norris Comprehensive Cancer Center, Los Angeles (http://ccnt.hsc.usc.edu) and co-workers that tumour cells preferentially respond to zebularine when compared with normal cells.

What is epigenetic therapy?

For many years, the search for cancer therapy targets focused on the genetic changes associated with the transformation of normal cells into malignant cells. But it is now clear that disruptions in epigenetic mechanisms – modifications of DNA or histones that heritably alter gene expression without mutating DNA – are important in cancer. For example, genome regions that normally contain non-methylated cytidine residues become methylated in cancer, inappropriately switching off the expression of tumour suppressors and other genes.

Epigenetic therapy for cancer – the removal of aberrant epigenetic marks – is appealing because it should make cancer cells more sensitive to other therapies [2]. The story of epigenetic therapy starts in the 1960s, explains Jones, when Czechoslovakian chemists synthesized two cytidine analogues – 5-azacytidine and 5-aza-2′-deoxycytidine (decitabine) – for use as cytotoxic drugs. Initial clinical trials were unsuccessful but, says Jones, 'we found that these drugs induced cell differentiation and gene expression and in 1980 reported that they worked by inhibiting DNA methylation.'

For the next decade, epigenetic therapy attracted little attention but in the 1990s clinical trials of the approach were restarted and on May 19 2004, Pharmion (http://www.pharmion.com) got US FDA approval for 5-azacytidine (Vidaza™) for the treatment of myelodysplastic syndrome. Interest in decitabine (Dacogen™) is similarly keen [3], with MGI PHARMA (http://www.mgipharma.com) buying the worldwide license for its development from Supergen (http://www.supergen.com) on September 1 2004.

Enter zebularine

'Unfortunately, 5-azacytidine and decitabine are very unstable in aqueous solution,' notes Jones. By contrast, zebularine, another nucleoside analogue, has a long half life in water. Originally designed as a cytidine deaminase inhibitor, Jones and his colleagues discovered last year that zebularine inhibits DNA methylation.

The researchers now report that, like 5-azacytidine and decitabine, zebularine is incorporated into DNA where complex formation with DNA methyl transferases (DNMTs) depletes cellular DNMT activity, causing DNA demethylation. Consequently, genes, including those involved in cell-cycle control, are re-activated and cell growth is inhibited. Importantly, normal cells are less sensitive to zebularine's effects than are cancer cells [1].

'There is considerable interest in using demethylation agents clinically,' comments Robert Brown, Professor of Cancer Therapeutics at the University of Glasgow, UK (http://www.beatson.gla.ac.uk), 'and these results further support the use of agents like these.' But, note Brown and Robert MacLeod, Director of Biology at MethylGene Inc., Montreal, Canada (http://www.methylgene.com), the key issue remains: does zebularine provide a therapeutic advantage over 5′-azacytidine or decitabine? Experiments that directly compare all three drugs should answer this question, says MacLeod.

More than one epigenetic therapy

Nucleoside analogues are not the only agents with potential for epigenetic therapy [2]. MethylGene, for example, is just starting a Phase II trial of MG98, an antisense reagent that specifically inhibits DNMT1 [4], in combination with interferon α in metastatic renal cell cancer. Another promising demethylating agent, says Brown, is procainamide, a drug previously used to treat heart arrhythmias. And there are also drugs that target histone deacetylase, another epigenetic silencing mechanism. Here, the histone deacetylase inhibitor, suberoylanilide hydroxamic acid. from Merck (http://www.merck.com) is furthest along in development.

'With agents that inhibit methyl transferases and histone deacetylases moving rapidly towards the market, we should soon see the emergence of agents that target other epigenetic modifiers,' predicts MacLeod. And, stress

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MacLeod, Brown and Jones, epigenetic therapies are not just for cancer; they could be useful in any disorder where there is an underlying epigenetic problem, such as inherited syndromes in which genomic imprinting is faulty.

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Stapled peptide induces cancer cell death

Jo Whelan, freelance writer

A new technique known as hydrocarbon stapling could allow peptides from the key domains of natural proteins to be used therapeutically. Using the technique on a peptide involved in apoptosis, researchers have succeeded in destroying cancer cells in a mouse model of leukemia.

Apoptosis (programmed cell death) is controlled by a complex web of protein–protein interactions involving so-called 'pro-death' and 'survival' proteins. Loren Walensky and colleagues at the Dana-Farber Cancer Institute (http://www.dfci.harvard.edu) report taking a 'death domain' from a class of pro-apoptosic molecules called BH3-only proteins and optimizing it to kill human leukemia cells in living mice.

Stabilizing BH3

BH3-only proteins contain a peptide subunit called BH3, which is a key initiator of cell death. BH3 is an α helix: a coiled, spring-like shape with amino acids on its surface that bind to and inhibit anti-death proteins such as BCL-2, as well as activating pro-death proteins under certain circumstances. However, when BH3 is produced synthetically without its parent protein, its shape is lost and its functionality impaired.

To stabilize the peptide, Walensky applied the hydrocarbon-stapling technique developed by chemist colleague Gregory Verdine. Some amino acids in the natural sequence are replaced with synthetic ones bearing hydrocarbon 'tethers'. These link to form chemical 'staples', which reinforce the α-helical structure. The new peptide retains its biological activity and actually binds more strongly to the BCL-2 target, the researchers report [1].

Therapeutic window

Importantly, it also passes across the cell membrane. Mice with established human leukemia given i.v. doses of the peptide for a week had a median survival time of 11 days, compared with five days for untreated leukemic controls. Tumor expansion was halted in most of the treated mice and many showed tumor regression. Post-mortem examination found no obvious signs of damage to normal tissues.

'There may be a therapeutic window for BH3 molecules in cancers that specifically exploit cell death pathways for survival,' says Walensky. 'If you can inhibit an anti-death protein distinctly required for the cancer but not singly necessary for a normal cell's survival, you may be able to trigger cell death in the cancer while leaving normal cells unharmed.'

Different BCL-2 proteins are implicated in different cancers, but all contain α-helical BH3 domains. The team hope to produce a panel of stapled BH3 peptides to explore and manipulate apoptotic pathways in various types of cancer cell.

Mimicking protein–protein interactions

'This is a proof of concept that you can turn on the death-promoting proteins with a peptidomimetic,' says Steve Dowdy of the Howard Hughes Medical Institute at the UCSD School of Medicine, La Jolla, USA (http://medicine.ucsd.edu) whose perspective article accompanied the Walensky paper [2]. 'More broadly it shows that mimicking protein–protein interactions can be therapeutically beneficial in preclinical models, and we should be able to apply this in other pathways too.' He adds that more work to investigate potential toxicity and solve the problem of delivery will be needed before the approach can be tried in humans.

The α-helix motif occurs frequently in biologically important protein interactions, so stapling might allow peptides to be used as drugs in many different applications. 'If we could target protein interactions at critical biological control points using the natural sequence for a protein target, we might have a whole new set of tools to study and manipulate protein interactions within cells', Walensky says.

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