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Drug reactivates genes to inhibit cancer

Vida Foubister, freelance writer

Many tumour suppressor genes become inactivated in cancer cells, and a new drug has been found that appears to reverse this process in an animal model. Zebularine, which acts as an inhibitor of DNA methylation, significantly reduces the size of malignant tumours in mice [1]. Scientists at the University of Southern California/Norris Comprehensive Cancer Center (http://ccnt.hsc.usc.edu/) believe that zebularine holds promise as a therapeutic because it is the first DNA methylation inhibitor to reactivate silenced genes through oral administration.

DNA methylation inhibitors

Genes that have a role in the control of cell growth are often not expressed in cancer. Until recently, the field has focused on the mutation or loss of these genes. However, of late there has been considerable attention directed at the silencing of tumour suppressor genes, which can occur as a result of abnormal methylation at their promoter region [2].

'You have a perfectly good gene there and it becomes silent,' said Peter A. Jones, Director of the USC/Norris Comprehensive Cancer Center. 'This then gives you a therapeutic target because if you can reactivate the gene by reversing its methylation, you can turn it back on again.' Another silencing mechanism that has also come to the fore involves changes to the chromatin structure in the promoter region of tumour suppressor genes. These changes are being targeted clinically through the use of histone deacetylase inhibitors, including valproic acid and suberoylanilide hydroxamic acid (SAHA).

The focus on methylation has reawakened interest in two drugs, 5-azacytidine and 5-aza-2′-deoxycytidine, which initially went through cancer clinical trials without impressive outcomes. At the time, their function as DNA methyltransferase inhibitors was unknown and, therefore, the studies were not appropriately designed to inhibit methylation.

'These drugs have come back into the clinic and have very interesting and promising early results,' said Jean-Pierre Issa, Associate Professor in the Department of Leukemia at The University of Texas MD Anderson Cancer Center (http://www.mdanderson.org/). 'This, and the basic science rationale that if you could strip off the DNA methylation the gene underneath is perfectly fine, has prompted people in companies to start looking for other inhibitors of DNA methylation.'

Based on a recently completed Phase III trial, Pharmion Corp. (http://www.pharmion.com/) plans to file a new drug application with the US Food and Drug Administration (FDA; http://www.fda.gov) this year for 5-azacytidine, commercially referred to as azacitidine, as a treatment for patients with myelodysplastic syndromes. SuperGen (http://www.supergen.com) has begun Phase III clinical trials with 5-aza-2′-deoxycytidine or decitabine, a drug that Issa currently works with, for advanced myelodysplastic syndrome.

A fortuitous discovery

Despite the promise of azacitidine and decitabine, these compounds must be administered intravenously or subcutaneously. Thus, a search is under way for chemically stable analogues of these drugs that retain the ability to inhibit DNA methylation.

Zebularine – 1-(β-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one – was not

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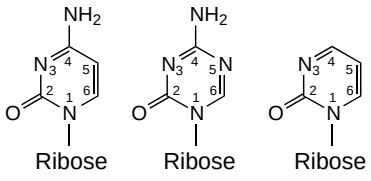
expected to be one of them (Fig. 1). 'The molecule doesn't look like it should inhibit DNA methylation,' Issa said. Instead, the ability of the drug to inhibit DNA methylation was discovered accidentally, when it was tested in conjunction with a potential methylation inhibitor for its known activity as a cytidine deaminase inhibitor. Although the trial drug failed to reactivate an antibiotic gene (*hph)* that is silenced by DNA methylation in the filamentous fungus *Neurospora crassa*, zebularine was found to be as effective as 5-azacytidine at reactivating this gene.

Scientists at the USC/Norris Comprehensive Cancer Center then tested its function both in mammalian cells in culture and in a mouse model. They found that zebularine inhibited DNA methylation and showed time-dependent reactivation of the silenced tumour suppressor gene p16 in T24 human bladder carcinoma cells. It was also effective when given to mice by either intraperitoneal or oral administration and had less toxicity than the known DNA methylation inhibitors in these experiments

Work is currently under way to determine the mechanism-of-action of zebularine. The current hypothesis is that it functions like 5-azacytidine and



Cytidine **5-Azacytidine** **Zebularine**

Drug Discovery Today

Figure 1. The structure of cytidine and two analogs, 5-azacytidine and zebularine, that inhibit DNA methylation. 5-azacytidine contains a nitrogen atom in position 5 and zebularine contains a 2-(1*H*)-pyrimidinone ring. Figure reproduced, with permission, from Ref. [1].

5-aza-2′-deoxycytidine, which are active only when incorporated into the DNA in place of cytosine. When DNA methyltransferase, an enzyme that specifically methylates cytosine bases that are followed by a guanosine base, attempts to methylate the fraudulent base it becomes trapped. 'Now you've got a state where you're making DNA but you have no active DNA methyltransferase in the cell so the DNA is hypomethylated,' said Jones.

Along those same lines, drug companies are working to develop small molecules that could bind to DNA methyltransferase without requiring DNA incorporation and 'thus inhibit it directly,' said Issa.

A potential limitation

Zebularine's promise as a therapeutic rests on its lower toxicity and stability when administered orally. One disadvantage, however, is that it requires high doses – up to 1 g kg^{-1} body weight in the mouse model – to achieve its inhibitory effect. 'In terms of drug development, that's really a huge amount of drug,' Issa said. 'It would surprise me if it could make it to the clinic that way.'

If these larger doses prove toxic in humans, another approach would be to use smaller amounts of zebularine in combination with other drugs, including 5-azacytidine and 5-aza-2′-deoxycytidine, or drugs that affect chromatin structure, such as the histone deacetylase inhibitors. 'There is great excitement at the possibility of combining the two approaches,' Jones said.

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