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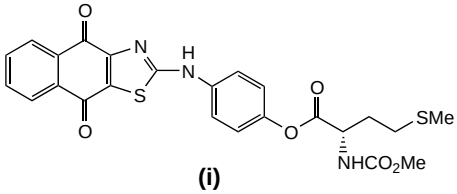
monitor

MOLECULES

Selective induction of apoptosis in Bcl-2-expressing breast cancer cells

Evasion of apoptosis is one of the fundamental 'hallmarks of cancer' [1], and aberrant regulation of apoptotic cell death is well-known to contribute to both tumour development and reduced sensitivity to chemotherapeutic agents. The Bcl-2 family, comprising both anti- and pro-apoptotic proteins, have important roles in caspase and apoptosis regulation, with high expression of the anti-apoptotic protein Bcl-2 being associated with a number of malignancies [2]. Inhibition of Bcl-2 or related anti-apoptotic proteins has been pursued as a strategy to either induce apoptosis in cancer cells or to sensitize these cells to chemotherapy; for example, the induction of apoptosis in a range of tumour cells has been demonstrated following administration of a Bcl-2 antisense oligonucleotide [3].

Structural analysis of the Bcl-2 protein [4] has facilitated the identification of small molecules that inhibit the interaction between the BH3 domain of pro-apoptotic proteins and the hydrophobic cleft of Bcl-2 or Bcl-x_L [5], and some of these compounds do have antitumour properties suggestive of therapeutic utility. However, the effect of Bcl-2-targeting agents on normal cells such as haematopoietic progenitors and epithelial cells has not previously been examined. Real *et al.* have recently reported the discovery of a new small molecule inhibitor of Bcl-2 [YC137; **(i)**] that induces apoptosis in breast cancer cell lines expressing high levels of Bcl-2 (e.g. MB435B and SUM159), by inhibiting the binding of Bid BH3 peptide to Bcl-2 [6]. Notably, the apoptotic response to YC137 was found to correlate with expression levels of Bcl-2 protein in a range of breast cancer cell lines. In contrast, various normal primary cells (e.g. $CD34^+$ progenitors, myoblasts and peripheral blood mononuclear cells) were found to be insensitive to YC137.



(i)

In vitro selection of cancer cells refractory to YC137 through generation of a YC137-resistant breast cancer cell line revealed reduced expression of Bcl-2, correlating with low activation of signal transducer and activator of transcription-3 (STAT3) and reduced expression of the human epidermal growth factor receptor-2 (HER2). Interestingly, YC137-resistant MB435B cells were found to be more sensitive to chemotherapy-induced apoptosis (paclitaxel or adriamycin). The elegance of this study lies in the selective killing of tumour cells expressing high levels of Bcl-2 through a small molecule Bcl-2 inhibitor, and in the enhanced susceptibility of resistant cells to conventional chemotherapy, suggesting a promising future avenue for rational combination treatments in the clinic.

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4 Petros, A.M. *et al.* (2001) Solution structure of the antiapoptotic protein bcl-2. *Proc. Natl. Acad. Sci. U.S.A* 98, 3012–3017
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MOLECULAR BIOLOGY

The NMR structure of a DNA-quadruplex inhibitor of HIV-1 integrase

HIV-1 integrase catalyses the integration of viral DNA into the host's genome and is essential for the lifecycle of HIV, making it a

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good target for anti-HIV drugs. It is inhibited by G-rich oligonucleotides, although the structure that these oligonucleotides adopt remains unclear. Phan *et al*. have solved the solution structure of the potent HIV-1 inhibitor *93del* and shown that it forms a stable quadruplex-DNA structure [1].

93del consists of the DNA sequence d(GGGGTGGGAGGAGGGT) and the authors showed that it forms a dimer in solution. The overall structure contained six planar d(GGGG) tetrads, with the central two tetrads containing G1 from one monomer and G2, G6 and G13 from the other, explaining the stable dimer formation.

The importance of the DNA sequence was analysed by altering the base or number of bases in the loops between the tetrads, or by deletion of the terminal bases. None of the substitutions significantly affected the NMR spectra, with the exception of removing G1, which presumably disrupted the dimer contacts. Interestingly, all of these sequences, including that lacking G1, inhibited HIV-1 integrase *in vitro*. The least inhibition was seen for sequences containing larger-loop substitutions, suggesting that it is the central quadruplex structure that is most important for inhibition. It will be interesting to see whether further alterations to the DNA sequence can be made to develop a more useful inhibitor.

The authors suggested a structure for the quadruplex DNA bound to HIV-1 integrase. HIV-1 integrase has been shown to form dimers and tetramers in solution. Tetrameric HIV-1 integrase forms a large positive channel that could accommodate a quadruplex DNA, although further research is required to firmly identify the binding site.

1 Phan, A.T. *et al*. (2005) An interlocked dimeric parallel-stranded DNA quadruplex: A potent inhibitor of HIV-1 integrase. *Proc. Nat. Acad. Sci. U. S. A*. 102, 634–639

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Hedgehog signaling becomes a prickly subject

The Hedgehog (Hh) signaling pathway is required for the proliferative phase during hair follicle cycling. It is also inappropriately activated in basal cell carcinoma (BCC). A recent study by Andrzej Dlugosz and colleagues has used a conditional mouse model to investigate the role of Gli2, a downstream effector of Hh, in the initiation and maintenance of BCC.

Skin-targeted expression of the Gli2 transgene was achieved in the absence of doxycycline. This led to formation of multiple BCCs, arising exclusively from the hair follicles. Administration of doxycycline to BCC-bearing mice inhibited expression of the Gli2 transgene. This in turn was associated with reduced expression of endogenous Hh target genes, decreased cellular proliferation, increased apoptosis and partial tumour regression. Morphologically, this process resembled the catagen phase of follicle development.

The residual BCC population was quiescent, similar to the stem cells found in the resting (telogen) hair follicle. In addition, the tumour cells expressed cytokeratins K1 and K10, and had the ability to form multiple differentiated lineages in a hair morphogenesis assay. However, growth could be reinitiated in the regressed tumours following reactivation of the Gli2 transgene.

This work raises several interesting points. First, do the non-proliferative, quiescent BCC cells represent a tumour stem cell? This is currently a hotly debated topic that cannot be easily addressed. Second, as aspects of the BCC growth and regression mirror those of hair follicle cycling, does BCC represent an example of cancer as an aberrant developmental process? Finally, the data suggest that using Hh signaling inhibitors to treat BCC might not be sufficient for total tumour regression. This therefore implies potential limitations for current therapeutic strategies.

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