Emerging molecular targets

p53 Tumor suppressor and cell growth

The protein dubbed p53 tumor suppressor sits at the apex of an intricate complex of intracellular pathways, some of which are yet to be fully defined, that regulate cell growth. It acts as a tumor suppressor either by inducing a transient arrest of cell cycling or by initiating apoptosis, leading to the death of an offending cell.

In human cancer cells p53 is frequently found in a mutated form, suggesting that an alteration of its function as a monitor of normal cell cycling is an important step in carcinogenesis. Evidence is accumulating to support the role of p53 as a transcriptional activator, and it is known to carry out its complex functions through interaction with cellular constituent molecules at multiple structural domains. Previously, four such functional domains have been identified as components of the p53 molecule. These domains trigger transcription, bind to sequence-specific and damaged regions of DNA, and act as sites of interaction during tetramerization.

Kristen Walker and Arnold Levine at Princeton University (Princeton, NJ, USA) recently generated a mutant form of the p53 protein in which proline-rich section 61–91 was deleted and assessed the role of the resulting p53 mutant protein as a tumor suppressor; the intact p53 protein has a total of 393 residues. Walker and Levine found that the mutant protein was no longer capable of suppressing the growth of tumor cells in culture. Deletion of residues 61–91 did not, however, block the ability of p53 to act as a transcriptional activator, suggesting that a functional activity in addition to transcriptional activation is essential for p53 to act as a tumor suppressor [*Proc. Natl. Acad. Sci. U. S. A.* (1996) 93, 15335–15340].

The authors of the study believe that the proline-rich domain encompassing residues 61–91 is a newly identified fifth functional domain of p53 involved in a signaling function that is critical for tumor suppression. They reached this conclusion because similar domains containing multiple repeats of the PXXP sequence in other proteins, where P is a proline residue and X is any amino acid, have been shown to bind SH3 domains of signal transduction enzymes. Additional evidence in support of the essential nature of this domain comes from analysis of the primary structure of p53 proteins from diverse organisms. The amino acid sequence of the region containing residues 61–91 has been highly conserved, suggesting it plays an important functional role.

Walker and Levine conclude that 'A reasonable hypothesis of how p53's proline-rich domain may signal is via contacting an SH3 domain of another protein. Indeed, both full-length p53 and the domain corresponding to residues 61–94 display SH3 domain binding activity *in vitro*.' The identification of p53's protein partner that binds to the newly identified fifth domain may lead to important new insights for drug discovery.

New targets for anti-obesity drugs

With the majority of the population in developed countries overweight if not obese, it does not take a genius to foresee that a safe, effective drug to control appetite would be a bestseller. In the US the current fad drugs in this market are a combination of phentermine and fenfluramine. The combination, affectionately known in weight-loss clinics as Phen/Fen, is an effective appetite suppressant. Fenfluramine appears to act by increasing the level of serotonin in the brain, while phentermine is an amphetamine.

However, recent positron emission tomography data from George Ricaurte's laboratory at Johns Hopkins University (Baltimore, MD, USA) suggest that fenfluramine at about twice the level needed for appetite suppression damages serotonin-containing cells in the brains of baboons [*Synapse* (1996) 24, 395–398]. This study supports the notion long held by neuroscientists that the use of Phen/Fen for appetite control may have long-term adverse consequences and hopefully will discourage its widespread use for weight loss.

But what's the alternative? A possible answer for a safe and effective appetite suppressant may be found in research emerging on the neuropeptide Y (NPY) receptor and the melanocyte-stimulating hormone (MSH). In a series of papers over the past few months it has been reported that both of these pathways in the brain play an important role, at least for mice, in feeding behavior. The NPY pathway appears to trigger feeding behavior upon weight loss while the MSH pathway appears to modulate feeding behavior in response to weight gain. An excellent summary of the new findings has recently been published [*Nature* (1997) 385, 119–120].

One of the most interesting recent findings is that by Wei Fan and coworkers at The Vollum Institute for Advanced Biomedical Research (Portland, OR, USA) and the University of Arizona (Tucson, AZ, USA). They followed up on speculation in the literature as to why mice with mutations at the agouti locus not only exhibit a novel bright yellow color but are also always obese. The mice are yellow because the peptide produced by the agouti locus inhibits pigmenting activity of MSH. It was suggested that the same mice are obese because the mutation may also lead to a malfunction of the MSH pathway in the brain [Lu, D. *et al. Nature* (1994) 371, 799–802]. Fan and coworkers used cyclic MSH analogs that act as agonists or antagonists of neural MSH to test the hypothesis. Sure enough, the feeding behavior of mice that had been fasted for 16 hours was inhibited by the MSH agonist and could be reversed by the antagonist. In addition, the elevated level of feeding induced by administration of the NPY peptide was also blocked by the MSH agonist [*Nature* (1997) 385, 165–168].

Perhaps, in this pathway, or the NPY pathway, a molecular target will be identified that leads to a drug to satisfy the high demand for a safe and effective appetite suppressant.

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HTS in *Monitor*

In a Special Report on pages 156–160, Dr Mark Rogers (Glaxo Wellcome R&D, Stevenage, UK) describes fluorescence-based assays, which are attracting considerable interest for HTS applications. In future issues, Dr Rogers will be contributing a regular current awareness column on HTS to *Monitor*.