Silent chromatin deadly for cancer cells

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The subcellular and molecular bases for cellular senescence, along with the specific protein pathway that activates the 'stop proliferating' response of human cells, have recently been revealed [1]. These important findings – from researchers at Cold Spring Harbor Laboratory (CSHL; http://www. cshl.org), State University of New York at Stony Brook (http://www.sunysb. edu) and the Curie Institute (http://www.curie.fr) – provide, for the first time, a clear explanation for the stability of the cell senescent state and shed new light on the action of tumour suppressors.

Mechanism revealed: silent DNA keeps cells in limbo

One mechanism to control cell division is apoptosis. Another is cellular senescence, which irreversibly arrests the growth of 'pre-cancerous' cells but allows them to remain alive and metabolically active [2,3]. This process emerges as a robust alternative to apoptosis in mediating cellular response to stress.

Previous research from the CSHL team, with collaborators from AntiCancer (http://www.anticancer. com), has shown that the disruption of senescence can promote tumour development or, in some settings, resistance to anticancer therapy [4]. By contrast, drug-induced senescence could contribute to successful cancer therapy by blocking the proliferation of cancer cells.

Now, the CSHL team has uncovered how cellular senescence keeps cancer cells on hold: oncogene-provoked cellular senescence involves the specific alteration of retinoblastoma tumour suppressor (Rb protein), which launches a chain of biomolecular events. These include the formation of a distinct nuclear structure of 'silent' DNA called senescence-associated heterochromatic foci (SAHF; Figure 1) and finally result in deactivation of the E2F target genes, a family of transcriptional regulators that control the expression of several genes involved in DNA synthesis [5].

Adam Lerner, an oncologist at Boston Medical Center (http://www.bmc.org), noted: 'Narita *et al*. [1] have identified an interesting and novel morphological/ biochemical correlate of senescence, specifically, SAHF, whose formation is shown to be uniquely dependent on Rb-mediated signaling. In addition, they demonstrate that such signaling is required for modifications of heterochromatin proteins associated with E2F-responsive promoters.'

Cellular senescence and tumour suppression

Senescent cells are not capable of expressing genes required for proliferation, even in a promitogenic environment. Accordingly, the CSHL researchers believe that their novel findings can be connected with potential therapeutic and/or diagnostic applications. 'Our new results provide some of the first insights into the effector processes leading to cellular senescence,' said Scott Lowe, Deputy Director of the CSHL Cancer Center, who led the study. 'As a consequence, they may eventually help us understand why chemotherapy works and fails, and suggest strategies to restore or activate senescence in cells in which the process has been lost,' he added.

Lowe continues: 'Our study essentially built a circumstantial case that the changes in heterochromatin were involved in maintaining senescence as a stable state. In this regard, it is provocative that the p16 and Rb tumour suppressors were crucial for these effects.' He also notes

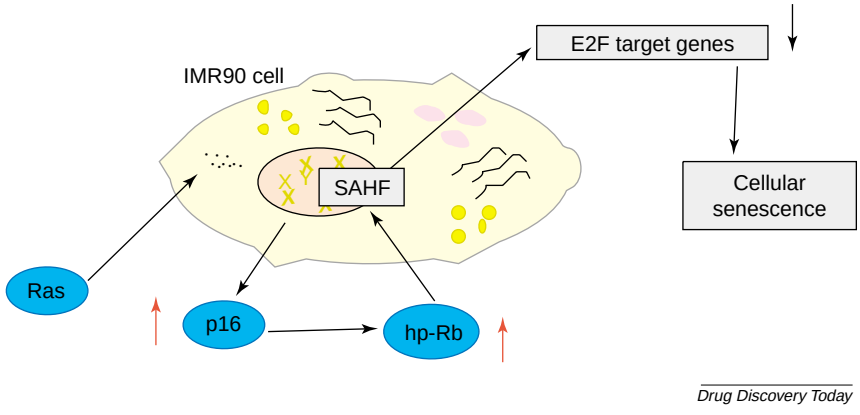

Figure 1. The Rb protein directs formation of senescence-associated heterochromatic foci (SAHF) and controls cellular senescence by silencing E2F target genes. Infection of IMR90 human fibroblasts with retroviruses expressing oncogenic *ras* makes these cells 'pre-cancerous' and stimulates the production of the p16 tumour suppressor [1]. In turn, this results in accumulation of an active, hypophosphorylated (hp) form of another tumour suppressor, Rb. These events coincide with the formation of SAHF, being associated with the stable repression of E2F target genes and, ultimately, with cellular senescence. Red and black arrowheads indicate protein accumulation and gene suppression, respectively.

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that, although the correlative data are compelling, they do not prove directly that the heterochromatin is the key determinant. Although it was proposed that the Rb suppressor acts directly by recruiting heterochromatin proteins to certain genomic regions, it is formally possible that Rb acts in the process indirectly.

'If cellular senescence happens not only *in vitro* but also *in vivo*, this work may have significant relevance,' says Manel Esteller, Director of the Cancer Epigenetics Laboratory at the Spanish National Cancer Center (CNIO; http://www.cnio.es). 'We know that the Rb and $p16^{INK4a}$ tumour suppressors are inactivated in many human tumours, mainly due to methylation-associated silencing. Thus, if both proteins are involved in cellular senescence, their inactivation may predict putative responses to certain chemotherapy agents,' Esteller continues. However, this is just a hypothesis that needs to be tested.

What next?

Scientists anticipate that the results from this study could have significant future implications for cancer diagnosis and treatment. Lerner notes that it will now be of great interest to examine, in residual primary tumour cells from patients who have undergone chemotherapy, whether SAHF and the chromatin changes described can be used as markers for cells that are alive but incapable of replication.

In contemplating ways of harnessing these new results, Lowe remarks: 'More information concerning two related issues needs to be obtained to know for sure: (1) to what extent does the senescence program we study in cell culture contribute to 'tumour suppression' or certain diseases in humans? (2) What are the more detailed molecular mechanisms of the process?' Lowe thinks that cancer genetics argues strongly for the importance of overriding senescence during tumour evolution, but there are no good 'markers' of senescence *in vivo*. He also thinks that the current information does not suggest obvious therapeutic targets, but with further study these might be identified.

However, Esteller argues that although the paper shows how the transition of certain human genome regions from active euchromatic to inactive heterochromatic state can be mediated by the Rb tumour suppressor gene, the Rb protein is known to trigger epigenetic changes, which cause gene silencing too. Also important is that these experiments were done with human fibroblast cell cultures and that it is not straightforward to translate such results to organs and tissues of the human body. 'We do not yet know how generalizable the results are, but this is certainly an important issue to resolve,' Lowe notes.

'There still exists a lot of concern that the senescence phenotype is a pure cell culture event and it does not occur in 'real life' or in primary human tumours,' adds Esteller. To this end, the Lowe's group hopes to resolve major uncertainties in future studies but for now most of these questions remain open.

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Bipolar disorder gene identified

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A single nucleotide polymorphism in the promoter of the G-protein receptor kinase 3 (GRK3) gene is associated with bipolar disorder (BPD) [1], reported US and Canadian researchers recently. 'Our paper provides the strongest, most definitive evidence for this gene's involvement in BPD to date,' explains John Kelsoe, Professor of Psychiatry at the University of California, San Diego (http://psychgenes.ucsd.edu/). Discoveries like this, he says, should lead to the development of rational drug therapies for BPD.

A complex genetic disease

BPD, which affects 1% of people during their lifetime, is characterized by extreme mood swings between mania and depression [2]. Family and twin studies indicate a genetic basis for BPD, but its mode of inheritance is complex, indicating the involvement of many genes in its aetiology, 'maybe as many as 10 or 20,' says Professor Peter McGuffin, Director of the Social, Genetic and Developmental Psychiatry

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