

Mechanisms of Cellular Senescence

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Our main research topic is stress-responsive phenotypes and tumour suppression. Of particular interest is cellular senescence, which involves multiple effector mechanisms, such as epigenetic regulation, DNA damage response, and a protein secretion program. Cellular senescence is a state of permanent cell cycle exit in response to cellular stresses, and is receiving increased attention particularly after a recent series of *in vivo* studies, which identified senescent cells in DNA damaged tissues, premalignant/benign tumours, and stem/progenitor compartments.

Cellular senescence and tumour suppression

Cellular senescence is an extremely stable state, where the cells are still viable and metabolically active. The stability of cell cycle arrest is important for its tumour suppressor function, but despite its importance the molecular basis is poorly understood. Using cellular differentiation – which is another state of stable cell cycle arrest – as an analogy, we have focused on epigenetic gene regulation as an effector mechanism of senescence.

Consistent with this idea, senescence is associated with global changes in chromatin structure, leading to the accumulation

of heterochromatin protein 1 (HP1) and histone H3 trimethylated on lysine 9 (me-K9H3) in senescence-associated heterochromatic foci (SAHFs) (Narita et al., *Cell* 2003; 113:703). Interestingly, SAHF formation is largely dependent on the p16/Rb tumour suppressor pathway, and has a close correlation with the silencing of cell-cycle genes and the 'irreversibility' of growth arrest in senescent cells.

Identification of senescence-associated chromatin factors

To further understand the molecular basis underlying the irreversibility of senescence arrest, we have been using a biochemical approach to analyse the alteration of chromatin protein profile during senescence. Using SDS-PAGE to visualize the protein composition of each chromatin preparation, we have identified HMGA proteins as new components of senescence (Narita et al., *Cell* 2006; 126:503). Considering the identification of HMGA proteins in this system as a proof of concept, we are currently taking a more systematic approach for a thorough analysis of chromatin protein profile in senescent cells. So far, we have several candidate proteins that specifically associate with chromatin to form senescent cells. Now we are in the process of verifying and analysing them in the context of senescence.

Are SAHFs an epitome of the dynamic chromosome regulation?

SAHFs have a very distinct chromatin structure and their formation can be acutely induced by oncogenic stimuli. Thus, SAHF formation has been successfully used as a read-out to identify new effectors in senescence and tumour suppression. Interestingly, we have shown that one of the SAHFs is an inactive X chromosome, and other groups recently suggested that each individual SAHF might represent each chromosome territory. Dynamically regulated chromatin alteration in a mammalian system would be useful to understand chromatin biology. Therefore, we are currently characterising SAHFs

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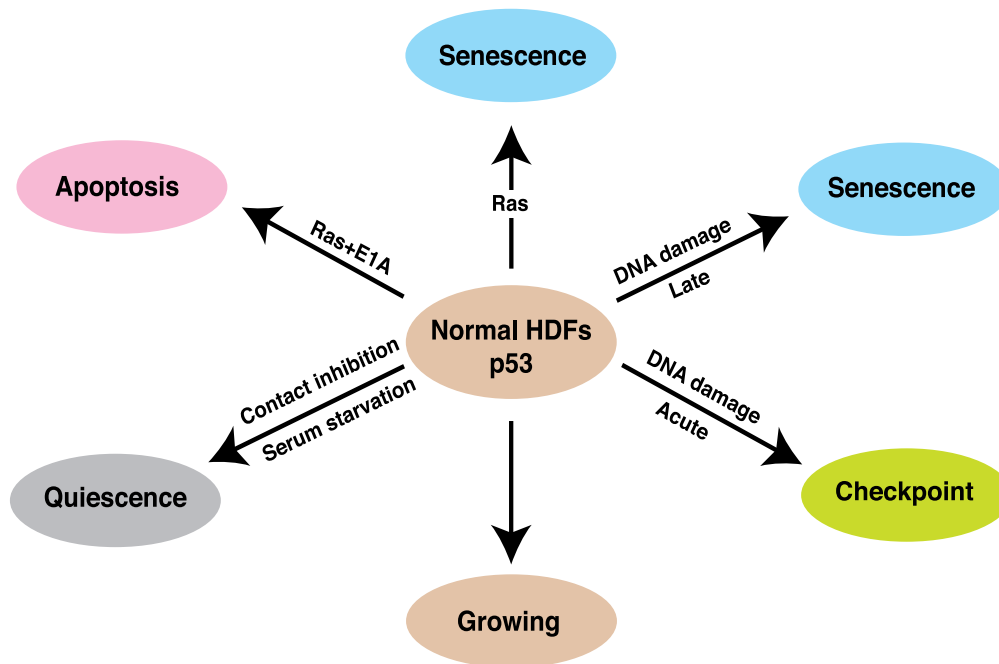


Figure 1. Model system for understanding a comprehensive picture of p53 functions. HDFs are genetically defined cells and we can induce indicated phenotypes by different triggers or at different time points. In each phenotype, p53 can be stably and acutely down-regulated by retroviral or lentiviral RNAi.

in more detail, and have found that SAHFs are composed of multiple layers based on specific histone modifications, rather than a simple heterochromatin structure. We are planning genome wide analyses of the individual layers during senescence to explore the functional relevance of the structure.

Identification of senescence-associated p53 function

A tumour suppressive transcription factor; p53, plays a critical role in many stress-responsive phenotypes, including DNA damage checkpoints, apoptosis, and senescence. Although ample data have supported a role for p53 in senescence, the precise mechanism is not clear. To address this issue, we are currently using normal human diploid fibroblasts (HDFs), where we can induce different phenotypes depending on environmental stimuli or other conditions (Figure 1). By adding either retroviral- or lentiviral-mediated stable RNAi to HDFs, we are comparing the impact of p53 knockdown on the gene expression profile in each condition, which represents a phenotype-specific p53 function. We have finished the array experiments, and are now attempting to build a comprehensive picture of p53's functions. So far, in a primary analysis, we have identified several genes whose products are upregulated in a p53-dependent manner during senescence, but not in the other stress responsive contexts (e.g. apoptosis). We are currently undertaking functional verification of one of the genes.

From epigenetics to proteins

Oncogene induced senescence (OIS) is a very dynamic process where cells typically undergo an initial burst of cell proliferation ('mitotic phase'), followed by the induction of pro-senescent factors, including p16 and HMGA2 ('transition

phase'). Eventually, the senescent phenotype dominates ('senescence phase') (Narita, *Br. J. Cancer* 2007; 96:686). During the transition phase, oncogenic and pro-senescence activities work against each other, and senescence usually prevails in normal cells. How cells can achieve such a drastic phenotype remodelling is unclear. A new area of interest in my group is in another layer of gene expression control, namely protein metabolism, during the senescence and transition phases. We reason that global epigenetic alteration should be coupled with efficient protein turnover as a part of the execution of epigenetic 'blue prints', in such an 'emergent' context (Figure 2). This project is currently ongoing.

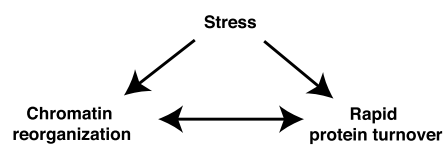


Figure 2. Concept of stress-responsive gene regulation.

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