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EPIGENETICS

Methylating patterns

> “senescence-associated methylation does not precede malignant transformation”

Global DNA methylation patterns are shared between replicative senescence and cancers, which has led to the hypothesis that tumour-promoting epigenetic patterns originate from cells escaping senescence. However, Xie et al. provide evidence for an alternative model whereby senescence-associated methylation does not precede malignant transformation and may even prevent tumorigenesis.

To observe DNA methylation changes during senescence and tumorigenesis, the authors used the classical transformation protocol of Weinberg in which early passage (EP) human BJ fibroblasts were successively infected with human telomerase reverse transcriptase (*TERT*), simian virus 40 large T antigen (SV40) and oncogenic $HRAS^{G12V}$. EP fibroblasts expressing the first two genes became immortalized whilst the addition of the third gene fully transformed cells, which were then capable of forming tumours as xenografts in mice. A second group of EP fibroblasts were infected with an empty vector and allowed to reach senescence naturally. Over time, this second group of cells entered a near-senescence state where after 14 population doublings, they displayed β-galactosidase staining, which is characteristic of senescence, but continued to proliferate before becoming fully senescent. DNA methylation patterns at the various stages during immortalization–transformation and senescence were then analysed. As expected, global gains and losses in DNA methylation were similar between transformation and senescence, yet at individual genomic regions the methylation changes were different. Specifically, changes at promoter CpG islands (CpGIs), gene bodies and enhancer elements were identical in near-senescence and senescence states, suggesting a defined early programme of methylation. This contrasted with the divergent stochastic methylation changes occurring early in $HRAS^{G12V}$-expressing cells. Intriguingly, the authors also found that oncogene-induced senescence (OIS) resulted in minimal DNA methylation changes, a finding which implies that cells that acquire oncogene addiction and facilitate tumour progression may be those that are selected to bypass OIS.

Examining the functional classes of genes with promoter CpGI hypermethylation during transformation and senescence, the authors identified a group of inducible development and differentiation genes enriched in the transformation state. These genes are normally poised for induction upon differentiation cues but aberrant cancer-specific promoter hypermethylation could promote the cancer cell phenotype by rendering these genes hard to induce. By contrast, the genes enriched in the near-senescent and senescent states are implicated in regulating biosynthetic and metabolic processes, intimating that silencing these genes could be necessary for slowing the metabolism of cells undergoing senescence.

Interestingly, a subset of hypermethylated genes that overlapped between transformation and senescence was also detected. These commonly methylated genes included developmental regulators and known cancer methylated genes. Importantly, both the commonly methylated genes and transformation-associated genes showed maximal gains in promoter hypermethylation in a range of primary tumour tissues compared with normal tissues, and an increased probability of being hypermethylated in peripheral blood mononuclear cells and skin samples from healthy older individuals, as well as in a panel of normal tissues wherein the gains in methylation track the curve of age-associated risk of cancer development. These observations propose that precursor cancer cells most likely evolve age-related DNA methylation patterns.

In additional experiments, the authors also demonstrated that near-senescent cells engineered to express *TERT*, SV40 and $HRAS^{G12V}$ are driven to bypass senescence and become immortal. However, these cells remain resistant to transformation and retain most of the methylation patterns observed in senescent cells.

Overall this study reveals how transformation induces a markedly different epigenetic state than that of senescence. The finding that the genes selected for by transformation-associated methylation patterns are related to ageing has implications for tracking cancer risk in individuals of every age group.

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