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> when *KRAS*^{G12V} was introduced ... CSC-exposed cells formed tumours *in vivo*

NON-SMALL CELL LUNG CANCER

Where there's smoke…

It is well-established that frequent smoking is associated with epigenetic changes in patients with lung cancer. These changes contribute to tumorigenesis; however, it is unknown exactly which role they play in tumour initiation and in what order they appear. A new study, recently published in *Cancer Cell*, demonstrates that chronic smoking elicits progressive changes in histone and DNA methylation patterns, which can sensitize lung epithelial cells to form tumours in response to a single oncogenic event.

To mimic chronic exposure to cigarette smoke, Vaz *et al.* treated immortalized, non-tumorigenic human bronchial epithelial cells (HBECs) with cigarette smoke condensate (CSC) for up to 15 months. After prolonged CSC exposure of 6–15 months, they observed that the DNA methylation pattern in HBECs had changed extensively. An increase in DNA methylation was found in regions close to transcription start sites, whereas a loss of DNA methylation was mainly found in regions within gene bodies. Interestingly, genes in which promoter regions were hypermethylated overlapped with those typically hypermethylated in human cancers and were involved in stem cell fate and cell fate decisions. Exploring the time course of events, the researchers found that these genes were marked by histone methylation and binding of enhancer of zeste homologue 2 (EZH2) in promoter regions after a short exposure of 10 days, followed by a switch to binding of DNA methyltransferase 1 (DNMT1) after prolonged CSC exposure. This switch happened in parallel with a progressive increase in DNA hypermethylation and a decrease in expression of the hypermethylated genes.

The phenotypic consequences were striking — even though no mutations were detected, key oncogenic signalling pathways such as KRAS–ERK, as well as WNT and epidermal growth factor receptor were strongly induced. Also, HBECs became pre-tumorigenic, featuring anchorage-independent growth and epithelial–mesenchymal transition. However, the cells could not form tumours in mice . The researchers explored whether CSC would sensitize HBECs, which can form tumours upon multiple oncogenic insults, to form tumours in response to a single oncogenic event, either by introducing mutant *KRAS* (*KRAS*^{G12V}) or by downregulating *TP53*. Indeed, when *KRAS*^{G12V} was introduced, they found that CSC-exposed cells formed tumours *in vivo*. These tumours had higher DNA methylation levels of CSC-specific genes and higher KRAS and WNT signalling activities. Additionally, analysis of patient-derived data from *The Cancer Genome Atlas* (TCGA) indicated that *KRAS* mutations and downstream signalling may be involved in maintaining smoking-induced aberrant DNA methylation even after the patients quit smoking, indicating a prolonged higher risk of developing lung cancer.

In summary, Vaz *et al.* provide direct evidence that smoking-induced epigenetic changes occur early in the course of tumour initiation, even before malignant transformation, and allow a single key oncogenic event to initiate the growth of a tumour.

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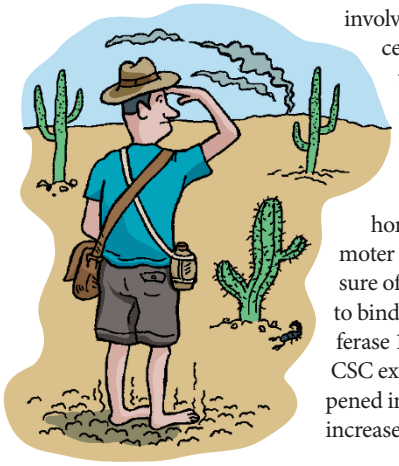

ORIGINAL ARTICLE Vaz, M. *et al.* Chronic cigarette smoke-induced epigenomic changes precede sensitization of bronchial epithelial cells to single-step transformation by *KRAS* mutations. *Cancer Cell* **32**, 360–376 (2017)