Nature Reviews Molecular Cell Biology | Published online 1 Sep 2016; doi:10.1038/nrm.2016.119

CANCER BIOLOGY

Hypoxia as an off switch for gene expression

DNA hypermethylation at promoters, which manifests with an increase in the levels of 5-methylcytosine (5mC), results in transcriptional repression. In cancer cells, DNA hypermethylation is often concentrated at the promoters of tumour suppressor genes owing to the impairment of demethylation, which occurs by unknown mechanisms. Thienpont *et al.* now show that oxygen deprivation (hypoxia) promotes DNA hypermethylation by interfering with the activity of ten-eleven translocation methylcytosine dioxygenases (TETs) — enzymes that are involved in DNA demethylation.

The first step of 5mC demethylation involves its TET-mediated oxidation to 5-hydroxymethylcytosine (5hmC). As this step involves oxygen, the authors reasoned that hypoxia, which is frequently associated with tumour expansion, might be linked to the impairment of DNA demethylation observed in cancer cells. Indeed, in hypoxic human and murine cell lines, global levels of 5hmC were decreased and this was linked to decreased TET activity resulting from hypoxia. The loss of 5hmC in hypoxic cells was most prevalent at promoter regions and it was associated with a concomitant increase in the levels of 5mC and a decrease in gene expression from the affected loci. Thus, hypoxia-induced impairment of TET activity directly translates to DNA hypermethylation with functional consequences for gene expression. Importantly, 5hmC levels were also decreased in cells from hypoxic regions of human tumours, which suggests that hypoxia drives DNA hypermethylation in cancers *in vivo*.

To further investigate the role of hypoxia in tumorigenesis, Thienpont *et al.* next analysed the DNA methylation and gene expression profiles for over 3,000 tumour samples in The Cancer Genome Atlas, confirming that DNA hypermethylation is strongly associated with tumour hypoxia; using statistical modelling, it was estimated that ~33% of all detected hypermethylation events were hypoxia-related. The products of hypermethylated genes were found to be involved in several processes, including cell cycle arrest, DNA repair and apoptosis, the downregulation of which could provide a strong advantage for cancer development.

Finally, these results were validated by manipulating tumour oxygenation levels *in vivo* in a spontaneous breast cancer mouse model. When hypoxia was induced in these mice, the generated tumours featured global loss of 5hmC with concomitant DNA hypermethylation, particularly of tumour suppressor genes. Conversely, promoting oxygenation by normalization of blood vessels prevented DNA hypermethylation of more-advanced tumours that are naturally prone to hypoxia.

In summary, this study uncovered a direct functional link between hypoxia and the regulation of gene expression through interference with 5mC demethylation, which induces DNA hypermethylation and consequently gene silencing. As demonstrated here, this hypoxia-mediated epigenetic regulation seems to be important during cancer development, but a similar mechanism might also be involved in other ischaemia-related human pathologies. Moving forward, it will be interesting to explore if and how this mechanism could be harnessed for therapeutic benefit.

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ORIGINAL ARTICLE Thienpont, B. *et al.* Tumour hypoxia causes DNA hypermethylation by reducing TET activity. *Nature* http://dx.doi.org/10.1038/nature19081 (2016)
FURTHER READING Pastor, W. A., Aravind, L. & Rao, A. TETonic shift: biological roles of TET proteins in DNA demethylation and transcription. *Nat. Rev. Mol. Cell Biol.* **14**, 341–356 (2013)