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CANCER

Suffocation of gene expression

If a tumour outgrows its blood supply, oxygen levels in its cells decrease. It emerges that this change can alter gene expression by limiting the activity of TET enzymes, which remove methyl groups from DNA.

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The addition of methyl groups to the DNA base cytosine leads to decreased gene expression, which has broad implications for embryonic development and tumour suppression[1]. Such methylation was once considered to be irreversible, but in 2009, it was found that ten-eleven translocation (TET) enzymes could catalyse DNA demethylation[2]. This discovery, fuelled by the finding that the gene *TET2* is frequently mutated in human blood cancers[3], sparked intense interest in understanding the function and regulation of this enzyme family. In a paper online in *Nature*, Thienpont *et al.*[4] report that TET activity is limited by oxygen supply — revealing a general mechanism by which gene expression can be silenced in solid tumours.

TET proteins belong to a dioxygenase enzyme family, members of which depend on three cofactors for their activity: divalent iron (Fe^{2+}), the metabolite α-ketoglutarate (αKG) and oxygen[5]. Fe^{2+} in the active site of the enzyme is coordinated by αKG to split an oxygen molecule into two oxygen atoms. One oxygen atom attacks and breaks a carbon–carbon bond in αKG, leading to the conversion of the metabolite to succinate and the release of carbon dioxide. The other atom oxidizes a carbon–hydrogen bond in the enzyme's substrate (Fig. 1). In TET-mediated reactions, methylated cytosine (5-methylcytosine, 5mC) is oxidized to 5-hydroxymethylcytosine (5hmC), and further oxidization follows, eventually leading to the removal of methyl groups and so to gene expression[6,7].

In addition to mutations that inactivate *TET* genes, TET enzymes can be inactivated in tumours if their cofactors are unavailable. For example, the accumulation of αKG competitors such as the metabolites 2-hydroxyglutarate (2-HG)[8,9], succinate and fumarate[10] causes decreased TET activity. The discovery[11,12] that these three metabolites accumulate in some tumours has led to the idea that cancer-promoting metabolites could have a general role in contributing to tumour development by altering the DNA-methylation landscape in cells, in much the same way that DNA damage causes cancer by altering the genomic landscape. Only a few types of cancer involve mutations in *TET* genes or show accumulation of αKG-competing metabolites. But the activity of TET enzymes — measured by the production of 5hmC — seems to be substantially decreased in a wide range of tumours[13]. This discrepancy has remained unexplained until now.

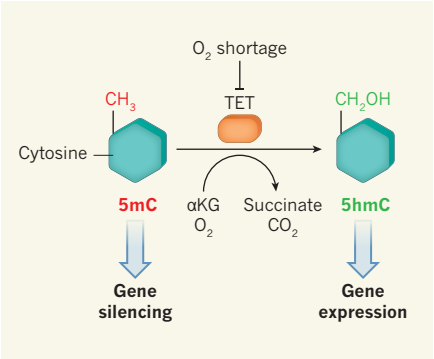

Figure 1 | Reducing TET activity through hypoxia. The addition of a methyl group (CH_3) to the DNA base cytosine to form 5-methylcytosine (5mC) can lead to silencing of many genes, including those that suppress tumour development. TET enzymes, acting with the cofactor molecules α-ketoglutarate (αKG) and oxygen, can trigger the demethylation of 5mC. In the first step of this reaction, O_2 is split into two atoms. One atom breaks a carbon–carbon bond in αKG, leading to succinate production and carbon dioxide release. The other oxidizes a carbon–hydrogen bond in CH_3 to form CH_2OH, converting 5mC to 5-hydroxymethylcytosine (5hmC), eventually leading to gene expression. Thienpont *et al.*[4] report that a shortage of O_2 in solid tumours inhibits TET activity, leading to DNA hypermethylation.

Solid tumours are oxygenated through blood vessels, but a tumour can rapidly outgrow its blood supply, leaving oxygen concentrations low in some regions. Thienpont *et al.* found that growing human or mouse cancer cells in such hypoxic conditions decreased 5hmC levels in some, but not all, of the cancer types they examined. Upregulation of *TET* gene expression could explain the cases in which no decrease was seen.

Why do 5hmC levels decrease in most cancer-cell types in hypoxic conditions? Damaging molecules called reactive oxygen species, which could impair TET activity by reducing the amount of Fe^{2+}, and metabolites that inhibit αKG such as 2-HG are known[14,15] to be increased by hypoxia. High levels of these molecules could therefore impair TET activity. However, the authors excluded both as the cause of TET inhibition — supplements of vitamin C, which counteracts reactive oxygen species, or of αKG could not prevent 5hmC loss. Instead, analysis of enzyme kinetics predicted a 45% decrease of TET1 activity and a 52% decrease of TET2 activity in typical hypoxic tumour cells in mice. This is the first evidence that oxygen molecules are a rate-limiting factor for TET2 activity in tumours.

Cytosine methylation typically occurs at CpG dinucleotide sites, where cytosine and guanine bases are found side by side. The authors analysed CpG methylation in a few tumours. CpG sites in most genes displayed increased 5mC levels that were concomitant with reduced 5hmC levels following hypoxia, suggesting a causal link between hypoxia and DNA hypermethylation.

To test this link further, Thienpont *et al.* turned to previously established gene-expression patterns known to be a signature of hypoxia[16], to assign tumours to hypoxic, normal or intermediate groups. The authors separately clustered the tumours into those that showed low, intermediate and high CpG methylation states. Hypoxic tumours predominated in the hypermethylated cluster, whereas normoxic tumours were enriched in the low-methylation cluster, providing further evidence that hypoxia leads to increased CpG methylation in tumours.

Thienpont and colleagues next found that hypoxia-linked 5hmC loss and concurrent 5mC gain were most apparent in promoter regions that drive gene expression — including the promoters of genes involved in DNA repair, the cell cycle, blood-vessel formation and cancer spread. Finally, the authors induced global loss of 5hmC *in vivo* by inducing hypoxia, and reversed this effect by deleting one copy of the oxygen-sensor gene *Phd2*, reduced function of which is known to restore tumour oxygenation[17]. Collectively, these results suggest that hypoxia causes TET inhibition, a reduction in 5hmC levels and DNA hypermethylation, leading to altered gene expression.

Oxygen shortage is unlikely to be the only factor that contributes to the widespread loss of 5hmC in tumours. 5hmC is a dynamic and

transient modification that could be affected by changes in *TET* gene transcription, by post-translational modifications of TET proteins or even by the dynamics of DNA methylation. Thienpont and colleagues' findings also raise the question of whether hypoxia could impair the activity of other dioxygenases that are dependent on Fe^{2+} and αKG, including those involved in DNA repair and in the demethylation of DNA-associated histone proteins.

This study also has clinical implications. Many conditions, from heart failure to stroke, can cause lasting oxygen shortage. Is TET activity impaired in these settings in ways that alter gene expression, contributing to disease progression? TET activity is frequently lost in solid tumours, but *TET* genes are rarely mutated. Could restoring TET activity in hypoxic tumours, for example by increasing levels of vitamin C, αKG or oxygen, reactivate tumour-suppressor genes that have been silenced by hypoxia-induced CpG hypermethylation?

In human tumours, drugs that inhibit blood-vessel formation have only incremental and variable benefits[18], probably in part because hypoxia contributes to tumour progression and treatment resistance. In some patients, paradoxically, this treatment leads to increased tumour oxygenation and is associated with longer survival. Perhaps making an informed selection of patients on the basis of each individual's TET activity and tumour methylation status could produce therapeutic benefits. Thienpont and colleagues' study reveals a new perspective from which to further investigate the regulation of TET and other dioxygenases that are dependent on Fe^{2+} and αKG in the development, and possibly therapeutic intervention, of hypoxia-related diseases. ■

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