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EPIGENETICS

# Methylation reboot

Whether epigenetic alterations can drive or maintain tumours is widely debated. However, apart from agents that cause global loss of epigenetic marks (for example, DNA methylation inhibitors), experimental tools to examine this question are lacking. Stefan Stricker, Steve Pollard and colleagues have used induced pluripotent stem cell (iPSC) reprogramming techniques to reset the epigenome of glioblastoma stem cells (GSCs) in order to investigate the contribution of epigenetic changes to malignant behaviour.

Using a panel of 14 GSC lines derived from independent primary human glioblastomas that already expressed high levels of MYC, the authors exogenously expressed the transcription factors Krüppel-like factor 4 (KLF4) and OCT4 (also known as POU5F1) to induce reprogramming. In two of the cell lines (G7 and G26), this resulted in the generation of iPSC-like cells (iG7 and iG26 cells) with transcriptional profiles more like those of iPSCs than those of normal neural stem cells (NSCs). These cells could form noninfiltrative teratomas (a property of pluripotent cells) when injected either subcutaneously or into the kidney capsule of non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice, but many of the teratoma cells expressed the neural progenitor marker Nestin and Ki67, suggesting that they were biased towards a neural lineage and remained proliferative. Interestingly, iG7 and iG26 cells also retain the structural chromosomal aberrations that are present in the parental G7 and G26 cell lines.

> “resetting of methylation marks is not sufficient to prevent tumorigenesis”

To determine what changes have occurred in the iG7 and iG26 cells, the authors examined DNA methylation patterns. Initial profiling of G7 and G26 GSCs compared with NSCs revealed 691 cancer-specific methylation variable positions (cMVPs), and the profiles of the GSCs were similar to those of glioblastoma generated by The Cancer Genome Atlas project. Importantly, a large proportion (450 of 691) of cMVPs in G7 and G26 cells was reset in iG7 and iG26 cells, and two known tumour suppressor loci (*CDKN1C* and *TES*) that are hypermethylated in GSCs were demethylated in the reprogrammed cells, as was a large proportion of Polycomb repressive complex 2 (PRC2) target genes. Therefore, it seems that abnormal cancer-associated methylation patterns are at least partially erased by the reprogramming procedure.

The functional consequences of methylation resetting were assessed using the G7 cells and iG7 derivatives, along with iG7 cells that had been directed to become neural progenitors (N-iG7 cells) or mesodermal progenitors (M-iG7 cells). More comprehensive methylation profiling revealed 60,977 cMVPs between normal NSCs and parental G7 cells; 44% of these were reset genome-wide (55% in regulatory regions) in the iG7 cells. When iG7 cells were differentiated to N-iG7 cells, 83% of the normalized cMVPs persisted. Despite the lack of many cancer-specific methylation marks in N-iG7 cells, these cells were able to form aggressive tumours that were indistinguishable from those formed from G7 cells following injection into the forebrain

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of NOD/SCID mice, suggesting that the resetting of methylation marks is not sufficient to prevent tumorigenesis. However, if the cells were directed towards a mesodermal lineage (M-iG7 cells) they were only capable of forming benign compact tumours in the mouse brain, suggesting that sending the iG7 cells down a different developmental pathway can suppress tumorigenesis.

Several interesting questions remain, such as how the reprogramming procedure affected other epigenetic changes and why some methylation abnormalities were not fully restored. Furthermore, it will be interesting to see whether this applies to other cancer types.

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**ORIGINAL RESEARCH PAPER** Stricker, S. H. *et al.* Widespread resetting of DNA methylation in glioblastoma-initiating cells suppresses malignant cellular behavior in a lineage-dependent manner. *Genes Dev.* **27**, 654–669 (2013)