HYPOXIA

A breath of fresh air

> expression of a set of hypoxia-induced genes is strongly indicative of poor prognosis

The association of hypoxia with cancer is two-way: tumours typically experience low oxygen levels that lead to a hypoxia response, and mutations that cause an inappropriate hypoxia response can lead to cancer. Now, Chi *et al.* have shown that expression of a set of hypoxia-induced genes is strongly indicative of poor prognosis.

Cells respond to hypoxia through the hypoxia-inducible transcription factors (HIFs). Under normal conditions the HIF α-subunit is degraded by polyubiquitylation, but in normal hypoxic conditions and in tumours the rate of degradation decreases.

The authors set out to define a hypoxia gene-expression signature in primary cultured normal cells to compare it with expression in tumour cells. Because the hypoxia response varies widely between cell types, they used several different epithelial and mesenchymal samples. They obtained expression profiles from each cell type at several time points over a 24-hour response to hypoxia. Clustering analysis showed classes of genes that were up- or down-regulated in response to hypoxia, some of which were common and some of which were cell-type specific.

As carcinomas are derived from epithelial cells, the authors used the data from the epithelial cell cultures to define a set of 168 genes that were consistently induced by hypoxia. Using several previously published data sets from breast and ovarian cancer they found that tumours with high expression of hypoxia-response genes were of higher grade, were more likely to have p53 and oestrogen-receptor deficiencies, and, most importantly, correlated with significantly shorter survival times.

For the simple analysis of new tumour samples, the authors defined a 'hypoxia-response score' from the average expression of the hypoxia-induced genes. This score was compared with other prognostic indicators using the data from one of the breast cancer studies. It was found to be a better indicator of survival than traditional factors such as age, tumour size, tumour grade or response to chemotherapy. The addition of the hypoxia-response score increased the prognostic power of a multivariate analysis by 10%. Also included in the analysis was a similar signature for the wound-healing response. The two signatures showed only a weak correlation with each other, and between them accounted for 40% of the predictive power of the analysis.

DNA METHYLATION

Methylation gastronomy

The bacterium *Helicobacter pylori* is bad news for the stomach — in particular because it is responsible for at least half of all gastric cancers. But how *H. pylori* causes cancer is not clear. In their recent paper, Maekita and colleagues explore the association between *H. pylori* and aberrant DNA methylation in the gastric mucosa.

The researchers measured the levels of DNA methylation at certain CpG islands of individuals who were, or who were not, infected with *H. pylori*. CpG islands are often aberrantly methylated in cancers, and in gastric cancer cells, tumour suppressors (specifically, *INK4a* (also known as cyclin-dependent-kinase inhibitor 2A, or p16), *CDH1* (also known as E-cadherin) and *MLH1*) are inactivated by DNA methylation more often than they are through mutation.

Maekita and colleagues used methylation-specific PCR to determine levels of methylation in eight regions of the genome — two regions in a CpG island associated with *INK4a* and six regions in CpG islands associated with *LOX* (lysyl oxidase, which is also a tumour suppressor), HRAS-like suppressor and *THBD* (both of which are putative tumour suppressors), and *p41ARC* (also known as actin-related protein-2/3 complex, subunit 1B), *HAND1* and *FLNC* (which are frequently methylated in gastric cancers).

They found that individuals who were infected with *H. pylori* (but who did not have gastric cancer) had high levels of abnormal methylation at all eight CpG islands. The same CpG islands were also aberrantly methylated in patients with gastric cancer (but no existing *H. pylori* infection). From this, the authors suggest that *H. pylori* might increase the risk of cancer by inducing aberrant methylation in the gastric mucosa, and they discuss possible mechanisms for this. Previous studies indicate that *de novo* DNA methylation can be induced by cell proliferation, which is a feature of *H. pylori* infection. The researchers also speculate that the strong, chronic inflammation could be responsible. Whatever the mechanism, these findings indicate that measuring levels of methylation at specific CpG islands could be used as a mechanism of risk assessment for gastric cancer.

Jenny Bangham

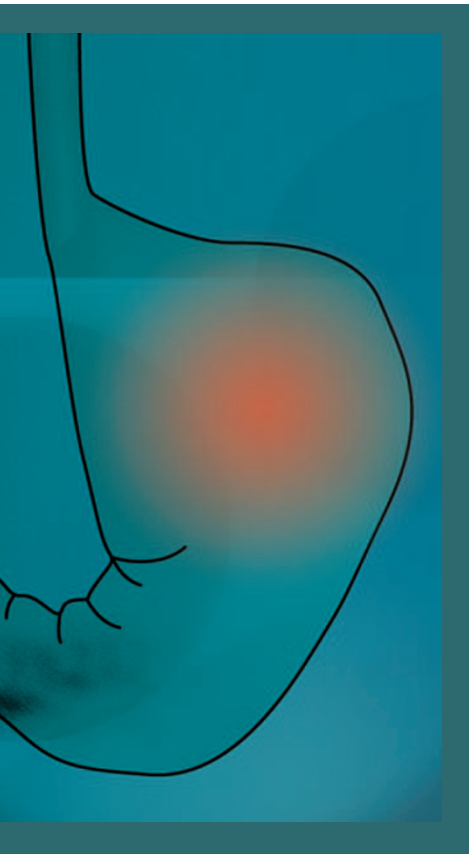
ORIGINAL RESEARCH PAPER Maekita, T. *et al.* High levels of aberrant DNA methylation in *Helicobacter pylori* — infected gastric mucosae and its possible association with gastric cancer risk. *Clin. Cancer Res.* **12**, 989–995 (2006)

> *H. pylori* might increase the risk of cancer by inducing aberrant methylation in the gastric mucosa

The nature of the observed hypoxia response in tumours and the precise mechanistic role that it has in affecting clinical outcome are still to be elucidated. However, this study is of considerable clinical importance and further demonstrates the power of hypothesis-led gene-expression studies for prognosis. Furthermore, such studies will help in identifying the causes and consequences of hypoxia responses in human cancers and in discovering whether the degrees of these responses correspond to the actual tumour oxygen tensions.

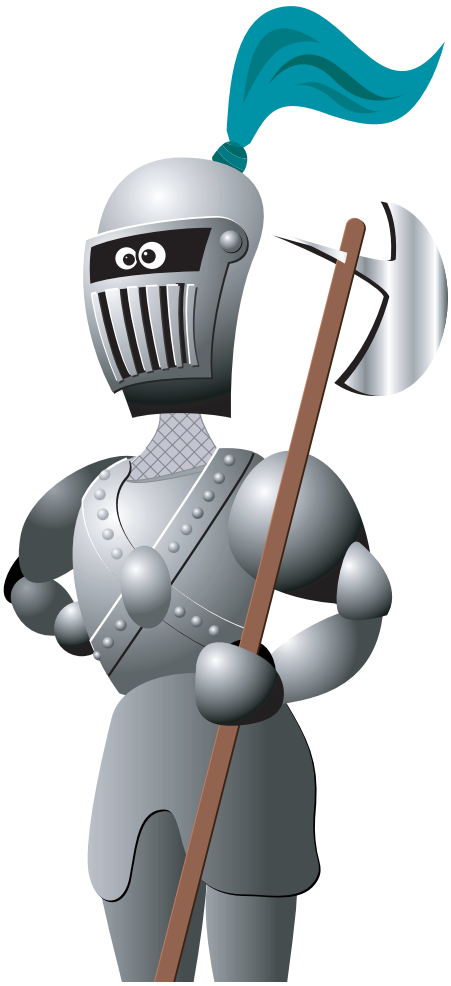
Patrick Goymer

ORIGINAL RESEARCH PAPER Chi, J.-T. *et al.* Gene expression programs in response to hypoxia: cell type specificity and prognostic significance in human cancer. *PloS Med.* **3**, e47 (2006)



GENOMICS

Complete coverage?



Does possession of the complete sequence of the human genome allow us to identify all the binding sites for a transcription factor? Until recently this has been logistically infeasible, but Wei *et al.* have now developed a technique to scan the entire genome for binding sites, and they have identified at least 98 previously unknown targets of the tumour suppressor and transcription factor p53.

Chromatin immunoprecipitation (ChIP) is a technique that extracts DNA fragments to which a transcription factor is bound. Having done this, these fragments need to be identified. One approach is to hybridize them to microarrays (ChIP on chip); this has been used successfully in yeast but mammalian genomes have proved too large. Alternatively, the fragments can be sequenced, but this has also proved unwieldy for mammalian genomes and only a few chromosomes have been studied in any one screen.

These authors have recently developed a new sequencing approach that is sufficiently efficient to screen the entire human genome — paired-end ditag (PET) sequencing. In this method, after cloning the precipitated fragments, the 5′ and 3′ ends of several clones are concatenated for efficient sequencing. Each pair of ends is then mapped to the genome to identify a potential binding site.

The authors chose to test their new method on p53 because of its importance in cancer and because many of its transcriptional targets are already known. They screened colorectal cancer cells that had been treated with 5-fluorouracil to activate p53 expression. After a comparison with expression data they identified 122 genes that are direct targets of p53, 98 of which were novel. Interestingly, they found a difference in the position of the binding site between genes that were upregulated and downregulated and even identified a second p53-binding site in the promoter of a well known p53-target gene, *CDKN1A*.

Several of the newly identified p53 targets are involved in cell motility, which is interesting as p53 is involved in suppressing metastasis. To further test the clinical significance of the p53 targets the authors looked at their expression in 251 breast tumour samples, some of which were p53 mutants and some wild type. The expression of the p53 targets clearly distinguished between the two types: many p53-downregulated genes were expressed at higher levels in p53-mutant tumours, and *vice versa*. Among the interesting biomarker candidates was the anti-apoptotic BCL2A1, which was identified here for the first time as being repressed by p53.

A further consequence of this study was a refinement of the consensus p53-binding sequence. Screening the genome *in silico* with this sequence identified many more genes that are potential p53 targets. This might represent the complete set of potential p53 targets, whereas the results of the ChIP–PET screen are the genes that are actually regulated by p53 in colorectal cancer cells treated with 5-fluorouracil. Studies with ChIP–PET on other samples will show how the complete set relates to specific sets. ChIP–PET might therefore prove an important development both in cancer genomics and in genome biology in general.

Patrick Goymer

ORIGINAL RESEARCH PAPER Wei, C.-L. *et al.* A global map of p53 transcription-factor binding sites in the human genome. *Cell* **124**, 207–219 (2006)
FURTHER READING Ng, P. *et al.* Gene identification signature (GIS) analysis for transcriptome characterization and genome annotation. *Nature Methods* **2**, 105–111 (2005)