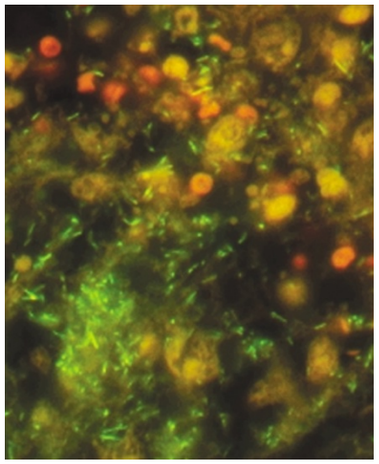
THERAPEUTICS

Taking a bite out of tumours



Clostridium novyi bacteria (green) destroying colorectal cancer cells (orange). Image courtesy of Long H. Dang and Bert Vogelstein, The Johns Hopkins School of Medicine, Baltimore, Maryland, USA.

Although many drugs are effective in killing rapidly dividing cancer cells, it is difficult to deliver these drugs to the poorly vascularized, hypoxic regions inside large tumours. Bert Vogelstein's group at Johns Hopkins University have developed an 'inside-out' therapy to destroy the oxygen-deprived necrotic areas of tumours — using meat-eating bacteria.

Although tumours induce formation of new blood vessels to deliver nutrients and oxygen to the growing tumour, angiogenesis does not keep pace with the growth of the neoplastic cells. This results in large hypoxic areas throughout the tumour. Cancer cells in these areas are not killed by ionizing radiation, which depends on oxygen, or by chemotherapeutic drugs, which do not reach these regions.

Reporting in *Proceedings of the National Academy of Sciences*, Long Dang *et al.* report an attempt to take advantage of the fact that necrotic tissues exist only in tumours and not in normal tissues. They performed a screen for anaerobic bacteria that can distribute throughout poorly vascularized regions of tumours. One of the strains they isolated, *Clostridium novyi* (which the authors grew on cooked meat particles) fulfilled this criterion, but was also, unfortunately, toxic to the mice. As this bacterium carries its toxin gene on a phage episome, the authors were able to isolate phage-free strains that no longer killed mice. But would these bacteria still be able to kill tumour cells?

The authors tested the ability of intravenously administered *C. novyi* spores to destroy colorectal tumours in a mouse xenograft model. The spores were administered with conventional chemotherapeutic agents, in the hope of killing the vascularized cancer cells located on the tumour's outside — as well as the hypoxic regions inside. Treatment with chemotherapy alone usually only slows, but does not stop, tumour growth. The combination bacteriolytic therapy (COBALT), however, caused the tumours to become black necrotic masses that shrank and disappeared, whereas surrounding normal tissue remained intact. More than three-quarters of the tumours treated, including very large tumours, were completely destroyed within 24 hours, and approximately half the mice were cured with no evidence of tumour regrowth. Similar results were seen with melanomas grown in C57BL/6 mice.

Interestingly, the authors found that co-administration of the bacterial spores with D10, a chemotherapeutic drug that acts by collapsing the tumour vasculature, was the most effective antitumour combination. The authors believe that the D10-induced vascular collapse further lowers the oxygen tension near the bacteria and increases their proliferation and activity.

CARCINOGENS

Fishing for trouble

Helicobacter pylori is a bacterium that is present in the gastrointestinal tract of about half the world's human population and is believed to cause gastric cancer. *H. pylori* strains carrying the *cagA* gene are known to be particularly virulent, but little is known about CagA function. Reporting in *Science*, Hideaki Higashi and colleagues now describe a protein-fishing expedition that led to the discovery of what might be a transformation-inducing CagA target.

***H. pylori* strains that carry the *cagA* gene are nasty creatures that attach to host gastric epithelial cells and inject CagA protein. Inside the cell, CagA is phosphorylated and triggers morphological changes similar to those induced by growth factors. But how can one bacterial protein activate cell shape change?**

To find out how CagA operates, Higashi *et al.* went fishing for proteins that interact with it. They transfected human gastric epithelial cells with haemagglutinin-tagged CagA, and also with a mutant form that could not be phosphorylated. As predicted, expression of wild-type, but not phosphorylation-resistant, CagA induced cellular elongation and spreading. Immunoprecipitation experiments revealed that the wild type — but not the phosphorylation-resistant — protein bound a protein called SHP-2. SHP-2 is a protein tyrosine phosphatase that is known to positively regulate signal transduction events from a variety of activated receptor tyrosine kinases. SHP-2 has also been shown to activate cell migration and adhesion.

SHP-2's phosphatase activity seems to be required for cell morphological changes. Treatment of CagA-expressing gastric epithelial cells with phosphatase inhibitors prevented induction of cellular spreading and elongation. Higashi *et al.* also created a phosphatase-defective form of SHP-2 that was still able to interact with CagA, and found that co-transfection of cells with this mutant, along with CagA, did not induce cell spreading.

So, if the phosphatase activity of SHP-2 is required to induce cell shape changes, what does CagA do? Because CagA is membrane associated, the authors proposed that the role of CagA might be to recruit SHP-2 to the plasma membrane. Accordingly, expression of a membrane-targeted, constitutively active form of SHP-2 was able to induce cell morphological changes in the absence of CagA. This indicates that SHP-2 acts at the plasma membrane to induce cell shape change, and its activation might be an important component of gastric tumorigenesis. It also provides an explanation for numerous epidemiological studies showing that *cagA*$^+$ strains are more carcinogenic than *cagA*$^-$ strains.

Kristine Novak

References and links

ORIGINAL RESEARCH PAPER Higashi, H. *et al*. SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* CagA protein. *Science* 2001 Dec 13; [epub ahead of print]
FURTHER READING Peek, R. M. Jr and Blaser, M. J. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. *Nature Rev. Cancer* **2**, 28–37 (2002)
WEB SITE
Hideaki Higashi's lab:
http://www.imm.hokudai.ac.jp/others/staff/staff-e.html

But can these bacteria be used to treat human cancers? Future experiments are required to answer safety questions, as the rapid destruction of large tumours was toxic and caused the death of some animals. It will also be important to determine the mechanism by which *C. novyi* selectively destroys viable tumour cells that are adjacent to hypoxic areas. The authors also point out that not all tumours will be susceptible to COBALT, and they will have to determine which chemotherapeutic drugs act synergistically with bacteria against human tumours.

Kristine Novak

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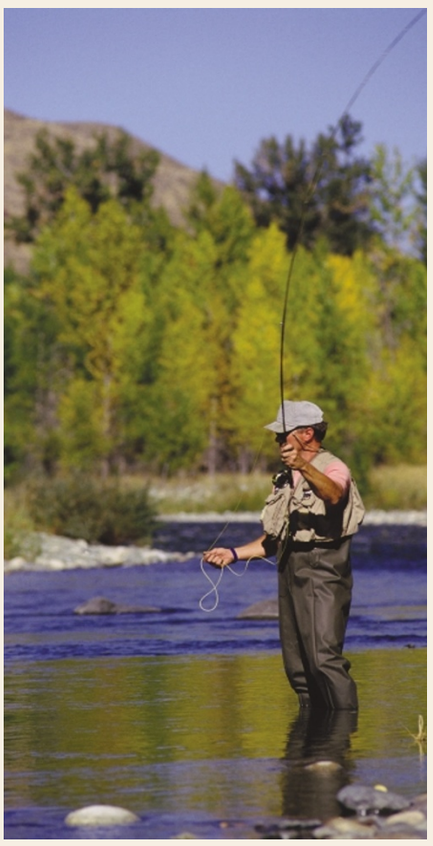
FURTHER READING Sznol, M. *et al*. Use of preferentially replicating bacteria for the treatment of cancer. *J. Clin. Invest*. **105**, 1027–1030 (2000)

WEB SITE

Bert Vogelstein's lab: http://www.med.jhu.edu/pharmacology/pages/faculty/vogelstein.html



GENOMIC INSTABILITY

Protecting the code

A code of post-translational modifications — including methylation, acetylation and phosphorylation — in the amino termini of histones determines the level of chromatin compaction. Histone acetylation is now recognized as an important therapeutic target in cancer, but what about other histone modifications? Thomas Jenuwein's group now provides the first indication that histone methylation might also influence tumour formation, by regulating genome stability.

Previous studies have indicated that the Suv39h enzymes — which methylate histone H3 at Lys9 — are required for correct chromosome segregation, so the authors generated knockout mice to investigate this function. Mice have two Suv39h genes — *Suv39h1* and *Suv39h2*. Both are expressed during embryogenesis, and *Suv39h2* is expressed in the testes of adult mice. Mice deficient in either Suv39h1 or Suv39h2 develop normally, presumably because the proteins act redundantly during embryonic development, but only a third of the expected number of double mutant mice (Suv39h dn) were born. Those that survived to birth were growth retarded and the males had underdeveloped testes. But are the Suv39h proteins required for chromosome segregation? A DNA content analysis of passaged primary mouse embryo fibroblasts (PMEFs) isolated from wild-type and Suv39h dn fetuses indicates that they are. After eight passages, wild-type PMEFs have largely senesced, but Suv39h dn PMEFs continue to proliferate and some have a polyploid DNA content. Interestingly, the chromosome morphology seems normal, indicating that whole chromosome sets are missegregated.

So, does the genomic instability induced in these mice increase susceptibility to tumour formation? Late-onset B-cell lymphomas — which most closely resembled slowly progressing non-Hodgkin's lymphomas in humans — developed in 33% of Suv39h dn mice, compared with none of the wild-type mice. Karotypic analysis of the lymphomas revealed that some had 'butterfly' chromosomes, which did not segregate as they remained attached at their centromeric regions. Could this be due to an effect on the pericentric heterochromatin region in these cells?

The authors used an antibody that acts as a marker of heterochromatin — by specifically recognizing H3 methylated at Lys9 — to show that both Lys9 methylation and heterochromatin were absent in Suv39h dn cells. But could modification of other histone residues that are known to be important for pericentric heterochromatin organization also be altered by lack of Lys9 methylation? A combination of immunoblotting and immunofluorescence revealed that phosphorylation of Ser10 in H3, and acetylation of Lys9 of H3 and Lys12 of H4, are affected by loss of Suv39h activity.

As mentioned, *Suv39h2* is also expressed in the testes, and Suv39h dn males have impaired spermatogenesis, characterized by the delayed onset of meiotic prophase and a subsequent increase in apoptosis. Interestingly, chromosome spreads show that there are many non-homologous interactions between chromosomes, predominantly between centromeres. This indicates a mechansim by which genomic stability is impaired in Suv39h-deficient mice.

So, the Suv39h methyltransferases comprise a potential new class of tumour suppressors that maintain genome stability by ensuring that the histone code is preserved in pericentric heterochromatin. The hunt is now on for loss of Suv39h function in human tumours.

Emma Greenwood

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ORIGINAL RESEARCH PAPER Peters, A. H. F. M. *et al*. Loss of the *Suv39h* histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell* **107**, 323–337 (2001)

WEB SITE

Thomas Jenuwein's lab: www.imp.univie.ac.at/groups/res.html/jenuwein