CARCINOGENESIS

The risk of hanging on

Long-term colonization of the stomach by *Helicobacter pylori* represents the highest known risk for the development of gastric adenocarcinoma, yet only a fraction of the many colonized individuals go on to develop cancer. Richard Peek and colleagues used a rodent model to show that bacterial adhesion to the host cells might be responsible for the increased risk.

The authors infected a gerbil with a human clinical isolate of *H. pylori* and allowed the bacteria to adapt to the host environment for 3 weeks. This produced a highly oncogenic strain, ideal for further study of the carcinogenic process. When a cohort of gerbils was infected with this strain, 75% developed gastric adenocarcinoma after 8 weeks, whereas no carcinoma developed following infection with the parental human isolate.

Using gastric epithelial cells *in vitro*, the authors found that their oncogenic strain increased the amount of β-catenin in the nucleus. In addition, a luciferase assay showed an induction of β-catenin-dependent transcriptional activation. These are key phenomena in the progression of many tumours, and can be caused by other carcinogenic agents. However, the authors were surprised to find that the mechanism by which *H. pylori* induces these changes is not the usual one of blocking the phosphorylation and ubiquitylation of β-catenin. Instead, they found that the *cag* set of pathogenicity genes from *H. pylori* was involved. These genes encode the molecular apparatus for transferring the CagA protein into the host cell, where CagA activates the host phosphatase SHP-2 to cause morphological changes. A series of genetic knockout experiments confirmed that CagA was indeed responsible for the oncogenic strain's pathological properties.

So, how does the oncogenic strain differ from its parent, given that both possess the *cag* genes and no other significant genetic gains or deletions were seen in a microarray comparison? Whereas both strains expressed CagA at similar levels, the oncogenic strain transferred it into the host more efficiently. This was

DRUG DELIVERY

A tiny timely vehicle

The administration of chemotherapy together with anti-angiogenic drugs seems to be a particularly effective way of slowing tumour growth. However, this combination also poses some practical problems — cutting off the tumour blood supply makes it difficult to achieve a high drug concentration, and hypoxia can trigger the expression of chemotherapy-resistance genes. Now, a group led by Ram Sasisekharan has designed a sophisticated delivery system that gets around these complications — a 'nanocell' that localizes to tumours and then shuts down the tumour vasculature before delivering a cytotoxic agent to tumour cells.

Their nanocell consists of a phosopholipid envelope and, inside it, a nanoparticle made of a biodegradable polymer. The researchers incorporated an anti-angiogenic agent — in this case combretastatin — into the liposome, and attached the chemotherapeutic agent doxorubicin to the nanoparticle.

They found that combretastatin escapes rapidly from the lipid envelope, while the conjugated doxorubicin is freed more slowly, degrading into smaller, inactive fragments before breaking down further into free, active doxorubicin. These release kinetics correlate well with the effect of the nanocell combination on the tumour endothelium *in vitro* — the system caused the vasculature to collapse as early as 12 hours post-administration, and tumours to be completely ablated by 30 hours.

The authors tested the therapeutic efficacy of this system *in vivo* using mice with B16:F10 melanomas and mice with Lewis lung carcinoma. They compared the effects of sequential drug delivery using nanocells with several other treatments — one or both drugs delivered simultaneously in simple liposomes, nanocells containing doxorubicin alone or co-administration of doxorubicin-containing nanocells and combrestatin-containing liposomes. Animals treated with nanocells containing both drugs had a better tumour response than any of the other treatment groups. In fact, the increase in survival of mice given the drugs sequentially was about twice that of those given the drugs simultaneously.

Furthermore, the nanocells containing both drugs resulted in the lowest systemic toxicity of all of the treatments. This is probably because the cytotoxic agent is localized to the tumour so effectively — the researchers confirmed this by attaching the dye fluorescein to the nanocell and

shown to be the result of better adhesion of the bacteria to the host cell.

Some of these *in vitro* findings were tested back in the rodent model. Here, the authors found that nuclear localization of β-catenin was increased only in the early stages of infection by the oncogenic strain, implicating this as the crucial time for carcinogenesis. They also confirmed that β-catenin was found in the nucleus more often in epithelial cells harvested from humans infected with *cag*$^+$ strains than those infected with *cag*$^-$ strains or no *H. pylori* infection at all.

The results indicate that the increased risk of cancer in long-term *H. pylori* infections might be a consequence of selection pressure on the bacteria to adhere to the host cells in order to remain in the stomach. However, the precise genetic change involved remains to be identified.

Patrick Goymer

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WEB SITE

Richard Peek's lab: http://ddrc.mc.vanderbilt.edu/faculty/peek.html

measuring concentration of the dye in tumours and highly vascular organs. They speculate that the nanocell might be retained preferentially in tumours because tumour vasculature is 'leakier' than normal vasculature, and allows tumour tissue to absorb large particles.

How does the temporal delivery system elicit such a good response? The authors found that the nanocells containing both drugs caused higher levels of apoptosis than the other treatments, as well as the lowest expression of hypoxia-inducible factor 1α (HIF1α), which they attribute to the high concentration of doxorubicin inside the tumour.

So what next for this new system? The nanocells used in this study could be developed further by adding probes that target the tumour vasculature more specifically. The authors point out that temporal drug delivery could substantially improve the efficacy of existing drugs, thereby reducing the risk and time for developing new therapies.

Jenny Bangham

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Ram Sasisekharan's lab: http://web.mit.edu/tox/sasisekharan/

TUMORIGENESIS

(H)IFs and buts

Hypoxia-inducible factor (HIF) is implicated in the development of renal tumours in patients with the inherited cancer syndrome von Hippel–Lindau disease, but is HIF also important for the development of benign tumours of the adrenal medulla (phaeochromocytomas) in these patients? Results from William Kaelin and colleagues, published in this months' *Cancer Cell*, indicate that this is not the case.

Some von Hippel–Lindau (*VHL*) mutations cause renal cell carcinomas but not phaeochromocytomas, and some *VHL* mutations cause phaeochromocytomas but not renal cell carcinomas. Most, but not all, *VHL* mutations disrupt the regulation of HIF, so is HIF disruption important in phaeochromocytoma development? Intriguingly, patients with germline mutations in the succinate dehydrogenase subunit genes (*SDHB*, *SDHC* and *SDHD*), in neurofibromatosis 1 (*NF1*) and in *RET*, also develop phaeochromocytomas. *SDH* mutations can increase HIF expression, but HIF has not been implicated in NF1- or RET-mediated tumorigenic pathways.

Phaeochromocytomas are comprised of chromaffin cells, derived from sympathetic neuronal progenitor cells. These cells are normally subject to selection during embryogenesis. Cells that make productive synapses acquire a supply of nerve growth factor (NGF) and survive and differentiate. Those that do not, die by apoptosis because of a lack of NGF-mediated survival signals. This death pathway is dependent on the expression of c-JUN. So, William Kaelin and co-workers decided to take a closer look at VHL function in rat phaeochromocytoma (PC12) cells. They found that expression of the c-JUN antagonist JUNB is increased by the loss of VHL. Transcription of *JUNB* is regulated by atypical protein kinase C (aPKC) family members. The activity of aPKC seems to be regulated by VHL, and the authors' results support the existing hypothesis that loss of VHL activates aPKC, which in turn activates *JUNB*. Since JUNB antagonises c-JUN-induced apoptosis on withdrawal of NGF, loss of VHL promotes neuronal survival by a JUNB-dependent mechanism. Importantly, JUNB regulation, but not HIF regulation, is universally altered by *VHL* mutations that predispose to phaeochromocytoma development. This indicates that JUNB, rather than HIF, is important in the VHL-mediated development of this tumour.

What about SDH mutant proteins and HIF expression in the development of phaeochromocytomas? Previous studies showed that NGF-induced apoptosis in PC12 cells involves the expression of a prolyl hydroxylase, EGLN3, which is related to the HIF-regulator EGLN1. The authors established that EGLN3 (but not EGLN1) was both necessary and sufficient for NGF-induced apoptosis and that EGLN3 did not affect HIF. Instead, inhibition of SDH function inhibits EGLN3 activity because the insufficient conversion of succinate to fumerate, which SDH catalyses, leads to a build up of succinate. Succinate inhibits EGLN3 and thereby blocks neuronal apoptosis.

The authors conclude that the development of phaeochromocytomas is because of a lack of developmental apoptosis in the neuronal progenitor population during embryogenesis, leaving neuronal cells that have the capacity to form these tumours. Germline mutations in *VHL* and *SDH* (and *NF1* and *RET*) all disrupt this process during development, giving rise to the increased risk of phaeochromocytoma seen in patients with these mutations. Despite the fact that both *VHL* and *SDH* mutations can affect HIF expression, HIF is not important in this context.

Nicola McCarthy

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William Kaelin's web site: http://www.hhmi.org/research/investigators/kaelin_bio.html