IN THE NEWS

To eat, or not to eat

The salmon-farming industry is in uproar over a comprehensive report in *Science* (9 January 2004) that shows that farm-raised salmon, particularly from Scotland and the Faroe Islands, contains more cancer-causing agents than wild salmon.

The study sampled 700 salmon that were bought in North America, South America and Europe and found that 13 organochlorine compounds were significantly higher in farmed Atlantic salmon than in wild Pacific salmon. "We think it is important for people who eat salmon to know that farmed salmon have higher levels of toxins than wild salmon from the open ocean" said the study leader Ronald Hites (*BBC News*, 8 January 2004).

Contamination with organochlorines — particularly polychlorinated biphenols (PCBs) and pesticides that contribute to the risk of liver and other cancers — could override the beneficial effects of eating this fish, which is a rich source of proteins, vitamin D and omega fatty acids, and can protect against heart disease and cancer. However, the chairman of the United Kingdom Food Standards Agency, Sir John Krebs, assures "This study shows that the levels of dioxins and PCBs in salmon are within internationally recognized safety limits. We advise that the known benefits of eating one portion of oily fish [per week] outweigh any possible risks." (*NewScientist.com*, 9 January 2004).

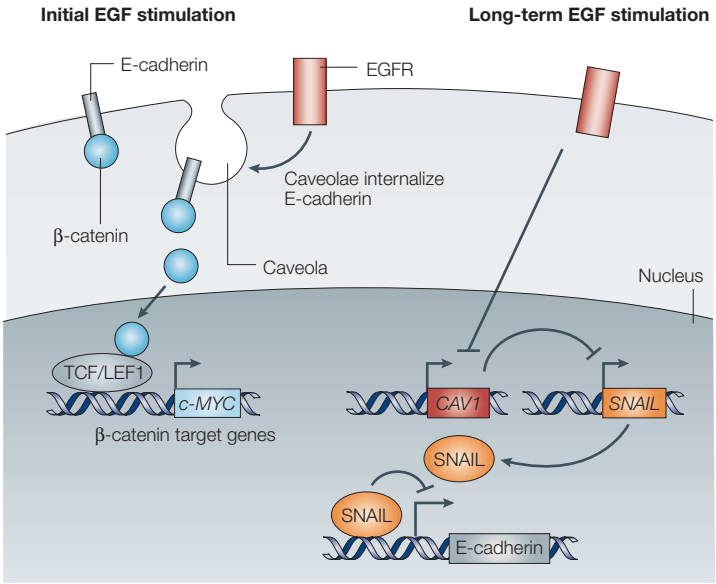
The study indicates that the contaminants come from the 'chow' that is fed to farmed salmon — a mixture of meal and oil made from other types of fish — thereby transferring toxins that have accumulated in the food chain. Steps are already being taken by the salmon-farming industry to find fish meal with low levels of contaminants.

Emma Croager

METASTASIS

CAV1 connection

During metastasis, changes in the tissue microenvironment allow cancer cells to escape the tumour and disseminate. Epidermal growth factor receptor (EGFR) overexpression and downregulation of E-cadherin — a key component of intercellular junctions — are both known to disrupt cell–cell interactions and, according to a report by Tony Hunter and colleagues in *Cancer Cell*, these processes are connected by the caveolae-associated protein caveolin-1 (CAV1). Not only does EGFR overexpression initially cause caveolin-dependent internalization of E-cadherin, it also ultimately reduces CAV1 protein levels, which activates the β-catenin signalling pathway and downregulates E-cadherin expression.



In response to EGF, EGFR-overexpressing cancer cells undergo an epithelial–mesenchymal transition, which is caused by loss of cell–cell junctions and cell depolarization. In A431 human cancer cells, rapid internalization of E-cadherin was associated with the early stages of this process and, as EGF is known to trigger CAV1 redistribution, the authors investigated whether the internalization was mediated by caveolae. Chemical depletion of cholesterol, a key component of caveolae, prevented E-cadherin internalization, indicating that caveolae are needed to downregulate surface E-cadherin and cause rapid loss of intercellular contacts (see figure).

E-cadherin normally associates with β-catenin — a component of the WNT signalling pathway — at intercellular junctions, and immunofluorescence experiments showed β-catenin was reduced on the surface of the EGF-treated cells.

THERAPEUTICS

All exits blocked

One way that tumour cells inactivate tumour suppressors is through sub-cellular mislocalization. For example, in some cancer cells, the forkhead (FOXO) family of transcription factors, which normally inhibit cell proliferation, are functional, but are exported from the nucleus to the cytoplasm. Pam Silver's group has developed a screen for compounds that prevent FOXO transport out of the nucleus and lead to cell-cycle arrest.

FOXO localization is regulated by the PI3K–PTEN–AKT signalling pathway. *PTEN* is mutated in several tumours, leading to loss of its lipid phosphatase activity and constitutive activation of AKT signalling. AKT phosphorylates FOXO transcription factors at several sites, which promotes nuclear export and thereby prevents their transcriptional activity. As studies showed that forcible localization of FOXO1A to the nucleus can reverse tumorigenicity of *PTEN*-null cells, Silver and colleagues developed a cell-based chemical screen for inhibitors of FOXO1A nuclear export. Using localization of FOXO1A as a visual assay, they screened over 18,000 compounds for their ability to relocalize FOXO1A to the nucleus in *PTEN*-null cells. Eighty nine compounds were found to cause FOXO1A nuclear retention, 42 of which were selected for further characterization.

Of these 42 compounds, 19 were also able to block export of the human immunodeficiency virus rev protein — another nuclear factor — and were therefore considered to be 'general export inhibitors'. These compounds were found to function by interfering with the activity of CRM1, an export receptor that shuttles proteins out of the nucleus. The other 23 compounds that were examined were only able to block nuclear export of FOXO1A, through several points along the AKT signalling pathway.

One of the compounds was identified as trifluoperazine, which acts as a calmodulin inhibitor, among other things. The authors therefore treated *PTEN*-null cells with other types of calmodulin inhibitor, and found that they all caused FOXO1A re-localization to the nucleus. This is the first report of a calmodulin-dependent regulatory mechanism of FOXO1A localization, and indicates that calmodulin could interact with the AKT signalling pathway.

So, the compounds that were identified in the screen cause FOXO1A to stay in the nucleus, but how do they affect tumour growth? Thirty of the nuclear-transport inhibitors that were discovered also

Moreover, β-catenin levels were increased in the nucleus of these cells, indicating that the release of β-catenin from the cell surface might result in nuclear translocation and increased transcription of β-catenin–TCF/LEF1 target genes. In fact, expression of c-*MYC* — a target of the β-catenin–TCF/LEF1 pathway — was increased in EGF-treated A431 cells and EGF increased activity of a β-catenin–specific luciferase reporter in EGFR-expressing 293T cells. So, EGF induces transcription of β-catenin target genes.

A secondary effect, caused by long-term exposure to EGF, was a marked reduction in *CAV1* and *E-cadherin* transcription in the A431 cells. Downregulation of *CAV1* by antisense RNA expression reduced E-cadherin levels in EGFR-expressing 293T cells by increasing expression of the SNAIL transcription factor, a known repressor of E-cadherin transcription. In addition, expression of antisense RNA to *CAV1* increased transcription of the β-catenin-specific reporter construct in EGFR-expressing 293T cells, whereas wildtype *CAV1* expression inhibited both basal and EGF-induced reporter activity. So, downregulation of CAV1 is required for EGF-induced β-catenin transcriptional activity. Inhibiting EGFR signalling in A431 cells reversed the effects of epithelial–mesenchymal transition and the cells formed tight cellular adhesions and expressed CAV1 and E-cadherin, confirming that the EGFR has a crucial role in downregulating these two proteins.

Finally, *in vitro* collagen-gel assays were used to determine whether CAV1 has a role in EGF-mediated tumour-cell invasion. Antisense RNA to *CAV1* significantly increased invasion of A431 cells in the absence of EGF and this effect was increased by EGF-treatment. This work provides an important insight into how CAV1 governs EGF-mediated tumour-cell invasion and forges a link between the EGF and WNT signalling pathways.

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References and links

ORIGINAL RESEARCH PAPER Lu, Z., Ghosh, S., Wang, Z. & Hunter. T. Downregulation of caveolin-1 function by EGF leads to the loss of E-cadherin, increased transcriptional activity of β-catenin, and enhanced tumor cell invasion. *Cancer Cell* **4**, 499–515 (2003)

WEB SITE

Tony Hunter's lab: http://pingu.salk.edu/~hunter

blocked proliferation of the *PTEN*-null cancer cells. Additional studies are required, however, before transport inhibitors can be used as cancer therapies. The drug LMB, which blocks CRM1-mediated nuclear export, has already been found to be highly toxic in Phase I clinical trials. So it will be important to learn whether this toxicity is linked to its effects on CRM1 or to its other possible activities. Nonetheless, the authors hope that some of the inhibitors that were identified in this screen might be developed as leads for new anticancer drugs.

Kristine Novak

References and links

ORIGINAL RESEARCH PAPER Kau, T. R. *et al.* A chemical genetic screen identifies inhibitors of regulated nuclear export of a Forkhead transcription factor in PTEN-deficient tumor cells. *Cancer Cell* **4**, 463–476 (2003)

FURTHER READING Kau, T. R., Way, J. C. & Silver, P. A. Nuclear transport and cancer: from mechanism to intervention. *Nature Rev. Cancer* **4**, 106–117 (2004)

WEB SITE

Pamela Silver's lab: http://research.dfci.harvard.edu/silverlab/

IN BRIEF

CHROMOSOME INSTABILITY

Dual roles of human BubR1, a mitotic checkpoint kinase, in the monitoring of chromosome instability.

Shin, H.-J. *et al. Cancer Cell* **4**, 483–497 (2003)

The mitotic checkpoint prevents chromosome instability by delaying anaphase until all chromosomes are properly attached to the mitotic spindle. Shin *et al.* show that BubR1, a component of the mitotic checkpoint machinery, is significantly reduced in cancer cells causing polyploidy. Its expression triggered apoptosis in polyploid cells and inhibited growth of polyploid tumours in mice, indicating that loss of BubR1 contributes to tumorigenesis.

METASTASIS

The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma.

Tian, E. *et al. N. Engl. J. Med.* **349**, 2483–2494 (2003)

Multiple myeloma (MM) cells metastasize to bone and produce osteolytic lesions, by shifting the normal balance between osteoblasts, which control bone formation, and osteoclasts, which control bone resorption. Tian *et al.* identified four genes that were overexpressed in plasma cells from patients with osteoclastic lesions, including Dikkopf1 (*DKK1*) — a secreted factor that inhibits skeletal development. DKK1 was detected in MM cells and inhibited the differentiation of osteoblastic precursors *in vitro*. So, by blocking osteoblast differentiation, DKK1-expressing MM cells promote osteoclast proliferation and osteolysis.

GENE EXPRESSION

Highly expressed genes in pancreatic ductal adenocarcinomas: a comprehensive characterization and comparison of the transcription profiles obtained from three major technologies.

Iacobuzio-Donahue, C. A. *et al. Cancer Res.* **63**, 8614–8622 (2003)

Iacobuzio-Donahue *et al.* combined data obtained from oligonucleotide gene arrays, complementary DNA arrays and serial analysis of gene expression to identify genes that are highly expressed in pancreatic cancer. This approach identified robust changes in gene expression and produced a set of six genes that might prove to be clinically useful for pancreatic cancer.

GENE THERAPY

Gene therapy insertional mutagenesis insights.

Davé, U. P., Jenkins, N. A. & Copeland, N. G. *Science* **303**, 333 (2004)

Three years after retroviral gene therapy cured nine children with X-linked severe immunodeficiency, two of the children developed T-cell leukaemia, which was caused by integration of the retrovirus near the known T-cell oncogene *LMO2*, which increases its expression. Davé and colleagues now show that the interleukin-2 receptor G gene, which is contained in the retrovirus, also has a role in the development of leukaemia, providing a genetic explanation for the high frequency of leukaemia that is observed in the gene-therapy trials.