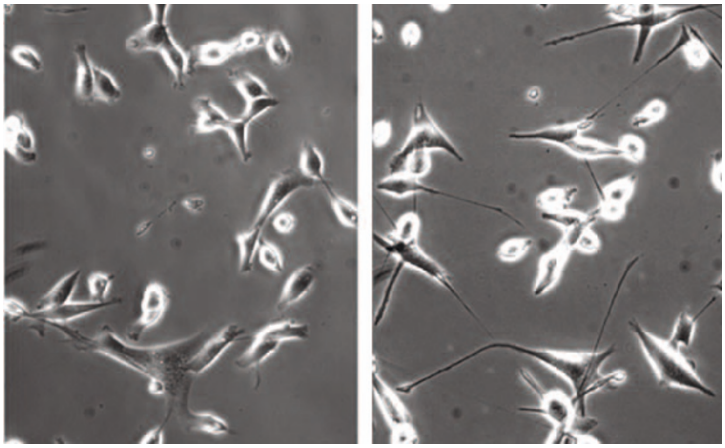

Treatment with D4 aptamer (left) inhibits RET-induced differentiation of NIH-3T3 cells (controls, right).

THERAPEUTICS

Holistic design

The promise of specific oligonucleotide ligands (aptamers) as potential regulators of biological processes is beginning to be realized, with several lead compounds in clinical trails. Laura Cerchia and colleagues now validate an aptamer selection protocol that targets transmembrane receptors in the form in which they exist on the surface of cells. The aptamers produced bind specifically to the RET receptor tyrosine kinase and inhibit its downstream signalling effects.

RET is mutated in multiple endocrine neoplasia type 2A and 2B syndromes and in familial medullary thyroid carcinoma. The C634Y mutation in the extracellular domain causes constitutive activation of the receptor. The authors used a 'systematic evolution of ligands by exponential enrichment' (SELEX) procedure that was modified to isolate aptamers using the RET^{C634Y} mutant expressed on PC12 cells. The authors reasoned that this *in vivo* procedure would select aptamers that bound only to transmembrane receptors in their natural physiological environment.

Initially, they incubated a library of 2′-fluoropyrimidine RNAs with parental PC12 cells to remove aptamers that bind non-specifically to the cell surface. To select for aptamers that specifically bound the mutant receptor, the supernatant was incubated with PC12–RET^{C634Y} cells. Unbound sequences were washed off, and the bound winning sequences cloned. The resulting aptamers did not bind to a recombinant extracellular C634Y RET fragment, highlighting the strength of the authors' whole-cell approach.

The winning aptamers were screened for their ability to inhibit the signalling of the mutant receptor and several blocked phosphorylation of RET^{C634Y} and of its downstream effector extracellular signal-regulated kinase (ERK). Surprisingly, the best inhibitor (D4) also binds to the wild-type human RET that is expressed naturally on neuroblastoma cells. D4 blocks phosphorylation of wild-type RET and ERK following stimulation of the receptor by its ligand, glial-cell-derived neurotrophic factor (GDNF). The aptamer also inhibits RET-dependent changes in cell phenotype: the growth of neurites from PC12 cells expressing the wild-type RET receptor and activated with GDNF is blocked by D4, as are changes in morphology seen in NIH-3T3 cells following expression of the constitutively active RET^{C634Y} receptor (pictured).

So it seems that D4 is a highly selective aptamer for RET that can block the downstream effects of RET signalling on cell differentiation, and possibly transformation. The authors suggest that this differential whole-cell SELEX approach will be useful in the isolation of other lead therapeutic compounds and diagnostic cell-surface markers.

Helen Dell

References and links

ORIGINAL RESEARCH PAPER Cerchia, L. *et al.* Neutralizing aptamers from whole-cell SELEX inhibit the RET receptor tyrosine kinase. *PLoS Biol.* **3**, e123 (2005)

IN BRIEF

TUMORIGENESIS

DJ-1, a novel regulator of the tumor suppressor PTEN.

Kim, R. H. *et al. Cancer Cell* **7**, 263–273 (2005)

PTEN is a tumour suppressor that inhibits the phosphatidylinositol 3-kinase (PI3K) pathway that regulates cell survival. How PTEN activity is controlled is uncertain, but now Kim *et al.* show that the candidate oncoprotein DJ-1 is a suppressor of PTEN. Overexpression of DJ-1 in mammalian cells blocked PTEN-induced cell death and boosted expression of PI3K downstream effectors. The authors suggest that DJ-1 might be a useful prognostic marker, as non-small-cell lung carcinomas with increased DJ-1 have a poor prognosis.

EPIGENETICS

Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer.

Fraga, M. F. *et al. Nature Genet.* **37**, 391–400 (2005)

The focus of cancer epigenetics has mainly been on aberrant DNA methylation, but Fraga *et al.* now highlight the importance of histone modifications. They find altered histone H4 modifications in a range of cancer cell lines and primary tumours. Examining a mouse model of multistage skin cancer showed that the changes begin early in, and progress throughout, tumorigenesis. As histone-deacetylase inhibitors are currently being developed to treat cancer, defining the histone modifications that occur during tumorigenesis will be vital.

THERAPEUTICS

ON01910, a non-ATP-competitive small molecule inhibitor of Plk1, is a potent anticancer agent.

Gumireddy, K. *et al. Cancer Cell* **7**, 275–286 (2005)

Patients with high levels of Polo-like kinase 1 (PLK1) are less likely to survive than those with lower levels. PLK1 helps to regulate the mitotic spindle, and Gumireddy *et al.* identify a small-molecule inhibitor of PLK1, ON01910, that induces mitotic arrest and apoptosis in tumour cells by competing for the substrate-binding site of the kinase. ON01910 has little toxicity, markedly slows tumour growth and can work in synergy with several current chemotherapeutic agents.

CANCER SUPPRESSION

DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis.

Barkova, J. *et al. Nature* **434**, 864–870 (2005)

What is the nature of the anticancer barrier that constrains human cancer progression? Jiri Bartek and colleagues have found that markers of an activated DNA-damage response are commonly expressed in early precursor lesions, and are induced *in vitro* following the expression of oncogenes that deregulate DNA replication. Furthermore, a genome-wide assessment of tumour DNA indicates that human cells activate an ATR/ATM-regulated DNA-damage response before genomic instability and malignancy are apparent.