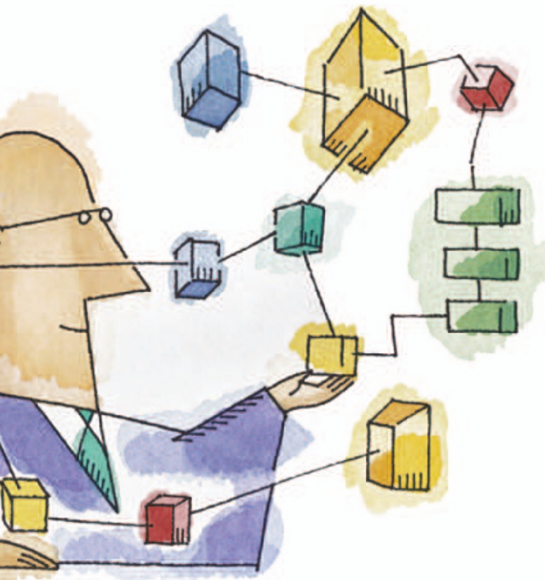


## TUMORIGENESIS

# The collagen connection



Patients with an inherited blistering skin disorder — recessive dystrophic epidermolysis bullosa (RDEB) — often develop epidermal squamous-cell carcinoma (SCC). Functional defects in type VII collagen are responsible for RDEB, and Susana Ortiz-Urda and colleagues have discovered that SCC development in these patients is dependent on collagen-VII-mediated interactions between the tumour and its microenvironment.

Intriguingly, a small subgroup of individuals with RDEB never develop invasive SCC, and the investigators wondered why, when all patients with RDEB have flaws in the same protein, some are susceptible to this cancer and others are not. Initially, they assessed the tumorigenicity of primary keratinocytes from patients with RDEB. The cells were transformed using oncogenic RAS and the NF- $\kappa$ B inhibitor I $\kappa$ B $\alpha$ , and then they were transplanted into immunodeficient mice. In this assay, cells from non-RDEB individuals form

epidermal tumours that appear the same as SCC. Tumours also grew from the keratinocytes from eight of the twelve patients with RDEB that were sampled, but not from cells from the remaining individuals with RDEB, confirming two distinct RDEB types — tumorigenic and non-tumorigenic.

The two populations could be further distinguished by the expression of collagen VII: primary keratinocytes from patients with tumorigenic RDEB express a portion of collagen VII, whereas the cells from the non-tumorigenic RDEB subset (known as RDEB<sup>Null</sup>) do not. Furthermore, expressing collagen VII in the RDEB<sup>Null</sup> cells restored their ability to form tumours. Ortiz-Urda and colleagues narrowed down the region of the protein responsible for tumour formation by expressing various fragments of collagen VII in RDEB<sup>Null</sup> cells. The sequence they identified (FNC1) restored tumorigenicity to RDEB<sup>Null</sup> cells, and antibodies against FNC1 prevented normal primary keratinocytes from forming tumours.

There are a range of neoplastic processes that FNC1 might affect — tumour-cell proliferation, survival and/or invasion — and the authors examined each of these. The FNC1

## ONCOGENES

# Trousseau's sign

The spontaneous formation of blood clots can be an indicator of neoplastic disease and was first described by Trousseau in 1865. Boccaccio and colleagues have now identified the genetic link between blood hypercoagulation and cancer.

The proto-oncogene *MET*, which encodes the hepatocyte growth factor/scatter-factor receptor, is known to be important for invasive growth during tissue morphogenesis and repair, and is also mutated in a number of human cancers. Boccaccio and colleagues expressed a constitutively active form of human MET under the control of the albumin promoter to limit its expression to mouse hepatocytes. Cancer most often arises from the transformation of somatic cells, so the authors used a self-inactivating lentiviral vector to target these cells in adult mice. A GFP-expressing lentivirus was used as a control.

*MET* was successfully expressed in the somatic hepatocytes, and nodules of dysplastic cells were evident after 3 months.

By this time, however, many of the *MET*-expressing mice had already developed blood clots, evident in their tails, and by 6 to 7 months, as a result of the exhaustion of the blood-coagulation system, many had haemorrhages in all major organs — a condition known as disseminated intravascular coagulation (DIC). Only 5 of 25 mice survived this and, of these, only a few went on to develop overt liver neoplasia.

Hepatocytes are important for the synthesis of a number of blood-clotting factors, so to rule out a hepatocyte-specific effect the authors expressed *MET* in mammary epithelial cells and transplanted these into the spleens of mice. These mice also developed DIC.

Microarray analysis has indicated that *MET* upregulates the expression of several genes associated with blood homeostasis. Indeed, the mRNAs encoding two factors, plasminogen activator inhibitor type 1 (PAI1) and cyclooxygenase-2 (COX2) are among the most upregulated of the 12,000 genes assessed. PAI1 inhibits fibrin

degradation and COX2 regulates platelet function, so the authors investigated whether the increased expression on these two proteins could explain the prothrombotic state. They found that PAI1 and COX2 were highly expressed in the *MET*-transformed mammary cells and were also expressed in the dysplastic liver nodules. Treatment of the mice with inhibitors of PAI1 or COX2 suppressed the onset of DIC, and, after prolonged treatment, the dysplastic liver foci also started to regress.

These data indicate for the first time that a single gene can both promote tumour formation in a small subset of cells and systemically affect blood coagulation by virtue of its effects on the expression of other genes. The therapeutic implications of these findings need to be carefully considered.

Nicola McCarthy

## References and links

**ORIGINAL RESEARCH PAPER** Boccaccio, C. *et al.* The *MET* oncogene drives a genetic programme linking cancer to haemostasis. *Nature* **434**, 396–400 (2005)

antibodies did not block tumour-cell division or trigger apoptosis. They did, however, halt invasion of normal keratinocytes, and restore the invasiveness of RDEB<sup>N<sup>ull</sup></sup> cells. Moreover, they stopped the expansion of tumours that were already established.

The FNC1 sequence contains a binding site for laminin-5, another extracellular protein that is found in SCC. Laminin-5 interacts physically with collagen VII, but the FNC1 antibodies disrupt this partnership. Moreover, it seems that laminin-5 is necessary for FNC1 to promote tumour invasion, as antibodies to laminin-5 inhibited this process.

Whether the presence of the FNC1 region in RDEB individuals is a reliable predictor that they will develop SCC has yet to be proven. But the authors suggest that as collagen VII and laminin-5 are extracellular, they will in principle be accessible to therapeutics such as antibodies.

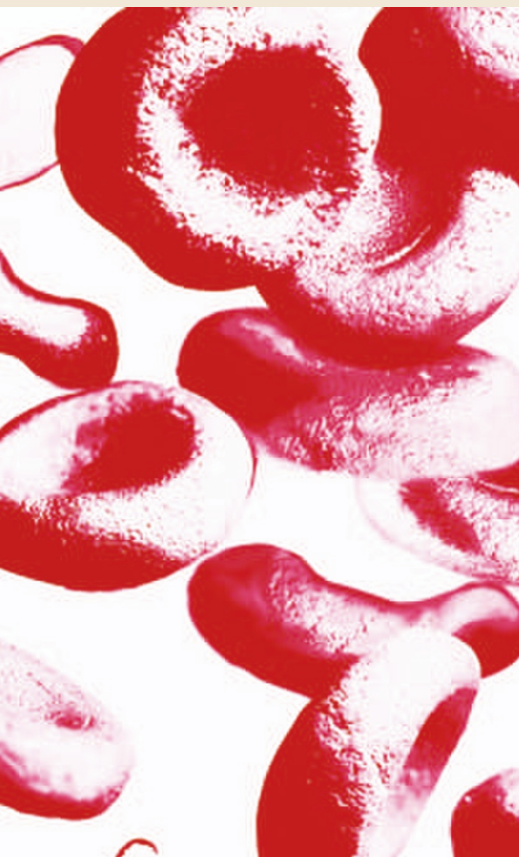
Helen Dell

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#### WEB SITE

Paul Khavari's lab: <http://cmgm.stanford.edu/khavari/>



#### TUMORIGENESIS

## Micromanager

MicroRNAs (miRNAs) are now recognized to be key regulators of development and cell differentiation. Unsurprisingly, therefore, evidence is emerging that mi-RNAs can be mutated or abnormally expressed in human cancers. Steven M. Johnson *et al.* now provide a link between certain miRNAs and the notorious RAS oncogenic signalling pathway, suggesting a mechanism by which these miRNAs might regulate tumorigenesis.

Animal miRNAs are proposed to exert their regulatory effects by binding to complementary sequences in the 3' untranslated regions (UTRs) of their target mRNA, thereby controlling translation. One of the first miRNAs to be discovered was *let-7* in the worm *Caenorhabditis elegans*, and there are now four *let-7* relatives known in these creatures. *let-7* controls cell fate in hypodermal seam cells, which are a type of epithelial cell, but the functions of the other family members are unknown. Johnson *et al.* searched the *C. elegans* genome for potential target genes, looking for sites complementary to a consensus *let-7* family sequence in the 3' UTRs of their genes. The highest-scoring candidate was *let-60*, the *C. elegans* homologue of human RAS.

The authors found that *let-7* and *let-60* are both expressed in hypodermal seam cells, but in a reciprocal manner, so that during developmental stages when *let-7* is high, *let-60* is low, and vice versa. Moreover, the *let-60* 3' UTR is sufficient to downregulate a reporter gene at the same developmental stage at which *let-60* expression normally drops off. Notably, in worms with *let-7* loss-of-function mutations, there was no decline in reporter-gene expression. All of which indicates that the *let-60* mRNA is a functional target of *let-7* in worms.

Moving into humans, Johnson *et al.* found several potential *let-7* complementary sites in the 3' UTRs of the three RAS genes. Transfecting HepG2 cells with a *let-7* miRNA reduced the expression of RAS by ~70%. Moreover, the 3' UTRs of *NRAS* and *KRAS* repressed the expression of a luciferase reporter in cells that have endogenous *let-7* (HeLa cells).

Co-transfection of an antisense inhibitor of *let-7* relieved this repression.



Several human homologues of *let-7* reside in genomic regions that are deleted in cancer, and Johnson *et al.* suggested that the loss of regulation by *let-7* miRNA could cause overexpression of RAS and contribute to tumorigenesis. They analysed levels of *let-7* in a range of cancers from 21 patients. In lung cancers, *let-7* was reduced by an average of half, compared with normal adjacent tissue, and northern and western analyses revealed the reciprocal correlation between expression of *let-7* miRNA and RAS protein. However, the amount of RAS protein did not seem to reflect the amount of *NRAS* mRNA, indicating that expression of human RAS protein is regulated significantly at the level of translation — consistent with a control mechanism where the miRNA binds to the 3' UTR. The authors concluded that *let-7* is strongly implicated as a tumour suppressor in lung tissue.

Helen Dell

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#### WEB SITE

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