

## CELLULAR IMMORTALITY

## Early old age



Werner syndrome is an autosomal recessive disease caused by inactivation of the gene encoding the DNA helicase WRN and is characterized by premature ageing, genomic instability and increased non-epithelial cancer incidence. It is thought that the erosion of telomeres — structures that cap chromosomes and are essential for chromosomal stability — has a role in the pathogenesis of this syndrome. Ron DePinho, Sandy Chang and colleagues have developed a mouse model that is null for both *Wrn* and the RNA component of telomerase (*Terc*) — an enzyme essential for telomere maintenance — that shows many of the classic features of Werner syndrome.

WRN is involved in DNA recombination, replication and repair, and hyper-recombination and numerous chromosomal aberrations have been observed in individuals with Werner syndrome. DePinho and Chang hypothesized that a combination of impaired DNA repair and telomere dysfunction might drive Werner syndrome pathogenesis.

The authors carried out successive intercrosses between *Wrn*<sup>-/-</sup> mice and *Terc*<sup>-/-</sup> mice to produce cohorts with progressively shorter telomeres and increasing telomere dysfunction. In first- and second-generation *Terc*<sup>-/-</sup> mice, *Wrn* status had no impact on clinical appearance, but the fourth- to sixth-generation *Terc*<sup>-/-</sup>*Wrn*<sup>-/-</sup> mice had lower body weights and shorter survival times than *Terc*<sup>-/-</sup>*Wrn*<sup>+/+</sup> mice. Although healthy in early life, by 12–16 weeks of age many of the *Terc*<sup>-/-</sup>*Wrn*<sup>-/-</sup> mice had features of premature ageing, including Werner-syndrome-related diseases. Increased apoptosis in gastrointestinal crypt cells and increased numbers of fused chromosomes in bone-marrow cells were seen in successive generations of *Terc*<sup>-/-</sup>*Wrn*<sup>-/-</sup> mice. This reinforced a link between genomic instability due to WRN loss and telomere dysfunction.

So, how did these genotypes affect the cancer phenotype of these mice? The prematurely aged late-generation *Terc*<sup>-/-</sup>*Wrn*<sup>-/-</sup> mice were

## IMMUNOLOGY

## Receptors and effectors

Effective therapies for non-Hodgkin's B-cell lymphoma aim to deplete the B-cell population in patients. However, the precise mechanism by which the humanized immunoglobulin G1 (IgG1) antibody therapy rituximab kills B cells was previously unknown. Jungi Uchida *et al.* now reveal the mechanism involved.

Rituximab, which targets a B-cell-specific antigen called CD20, could affect many aspects of the immune response, including antibody, effector-cell-(macrophage and natural killer cell) and complement-dependent cytotoxicity; the disruption of CD20 signalling pathways; and the induction of apoptosis. Previous studies have looked at the mechanisms *in vitro* or in circulating human B cells only. So, the authors developed a mouse model for anti-CD20 immunotherapy using 12 mouse anti-mouse CD20 monoclonal antibodies (mAbs) to study each of the possible mechanisms. All these antibodies bound to B cells in the CD20 wild-type mice and

depleted both the circulating and splenic B-cell compartments. The effectiveness of mAb-induced B-cell depletion correlated closely with mAb isotype — a single injection of an IgG2a mAb (MB20-11) depleted more than 95% of blood B cells and more than 93% of splenic B cells. None of the antibodies had any effect in *Cd20*<sup>-/-</sup> mice.

Immune effector cells express three different Fc receptor classes for IgG. FcγRI is the highest-affinity receptor and binding of IgG to it triggers phagocytosis by macrophages and cytotoxicity by natural killer cells. Although treatment of mice deficient in either FcγRI or FcγRIII with MB20-11 did deplete B cells, treatment of mice deficient in both FcγRI and FcγRIII did not deplete B cells. This shows that binding to one of these receptors is important for efficacy of anti-CD20 mAbs. Next, the authors looked at complement-deficient mice to assess the role of complement in B-cell depletion by anti-CD20 mAbs.

*In vitro*, the antibodies caused B-cell lysis and apoptosis only in the presence of complement. However, *in vivo*, there was no difference in the ability of any mAb to induce B-cell killing in the wild-type or complement-knockout mice.

So, Fc receptors are crucial for the efficiency of anti-CD20 mAbs; but what are the effectors of this response? When mice lacking T cells or natural killer cells were treated with MB20-11, more than 96% of B cells were depleted. However, similar treatment of macrophage-deficient mice did not cause significant depletion of circulating or splenic B cells.

The authors conclude that a likely mechanism of B-cell depletion by anti-CD20 mAbs is FcγR-mediated phagocytosis of mAb-coated B cells by macrophages. This knowledge should help to understand the response and resistance to rituximab therapy and the development of effective methods to enhance the benefits of therapies for non-Hodgkin's lymphoma.

Ezzie Hutchinson

 **References and links**

**ORIGINAL RESEARCH PAPER** Uchida, J. *et al.* The innate mononuclear phagocyte network depletes B lymphocytes through Fc receptor-dependent mechanisms during anti-CD20 antibody immunotherapy. *J. Exp. Med.* **199**, 1659–1669 (2004)

not particularly prone to cancer (presumably because they died before cancers developed), but the first- to third-generation *Terc*<sup>-/-</sup>*Wrn*<sup>-/-</sup> mice did have an increased incidence of osteosarcomas and soft-tissue sarcomas, usually developing at about 63 weeks. *Terc*<sup>-/-</sup>*Wrn*<sup>+/+</sup> mice developed tumours later (around 85 weeks), and these were mainly lymphomas.

The study of this compound-mutant model of Werner syndrome supports the view that WRN is involved in telomere dynamics and that inactivation of this protein forms the basis of ageing phenotypes that target slowly proliferating mesenchymal tissues.

Ezzie Hutchinson

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#### WEB SITE

Ron DePinho's lab: <http://www.dana-farber.org/abo/danafarber/detail.asp?PersonID=51&RD=True>



### METASTASIS

## Stick or twist?

For many years the similarities between the migratory properties of metastatic cancer cells and embryonic stem cells have been noted. In a recent *Cell* paper, Weinberg and colleagues now show that *TWIST* — a master gene that controls epithelial–mesenchymal transition (EMT) in embryogenesis — is required for metastasis in epithelial-derived breast tumours.

Weinberg and colleagues have exploited a breast tumour model that uses four spontaneously arising mouse breast tumour cell lines with distinctive metastatic properties. The four lines range from having no metastatic potential to being highly metastatic. By comparing microarray expression profiles of the primary breast tumours arising from each of these lines *in vivo*, the authors have identified genes that show altered expression patterns as metastatic potential increases. As well as identifying genes that are known to be involved in metastasis, such as the gene encoding matrix metalloproteinase 9, they also isolated *Twist* as the second most robustly upregulated gene when comparing metastatic with non-metastatic tumours.

To investigate the contribution of *Twist* to metastasis in this mouse model, the authors used short interfering RNAs to suppress expression of *Twist* in the most metastatic cell line. Suppression of *Twist* did not affect primary breast tumour formation, but did significantly suppress metastasis by inhibiting both the capacity of the cells to enter blood vessels and their ability to establish micrometastases within the lung.

How might *TWIST* influence the capacity of a cell for metastasis? *TWIST* is an evolutionarily

conserved transcription factor that regulates tissue reorganization during embryogenesis and, importantly, enables cells to migrate. Therefore, Weinberg and colleagues asked if *TWIST* might confer migratory properties to human mammary epithelial cells. Cells expressing *TWIST* developed a spindle-like, fibroblastic morphology, and had reduced cell–cell contacts, which correlated with reduced expression of adherens-junction proteins such as E-cadherin and  $\beta$ -catenin. This altered morphology is one of the hallmarks of cells that undergo EMT and is closely associated with migratory properties.

Is *TWIST* involved in human breast cancer? The authors compared the levels of *TWIST* expression in metastatic and non-metastatic human breast cancer cell lines and showed that only metastatic lines expressed *TWIST*. Moreover, microarray analysis of *TWIST* expression in specific breast cancer subtypes — ductal, mixed ductal/lobular and lobular carcinoma — revealed that 70% of the latter type expressed high levels of *TWIST*. Interestingly, lobular carcinoma cells show many of the hallmarks of EMT cells, prompting the authors to examine E-cadherin mRNA expression in these tumours — the level was substantially reduced. These findings correlate with the aggressive, invasive nature of lobular breast carcinomas.

*TWIST* is also overexpressed in diffuse-type gastric cancers, which also show infiltrative growth and reduced levels of E-cadherin. Therefore, the authors speculate that E-cadherin expression might be directly suppressed by *TWIST* and that this is one mechanism through which *TWIST* promotes invasive tumour growth.

Nicola McCarthy

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**ORIGINAL RESEARCH PAPER** Yang, J. *et al.* *Twist*, a master regulator of morphogenesis, plays an essential role in tumour metastasis. *Cell* 117, 927–939 (2004)

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#### WEB SITE

Robert A. Weinberg's Lab: <http://web.wi.mit.edu/weinberg/pub/>

