CANCER GENETICS

Women only

Women-only nights can be fun, but other events that occur exclusively to women are not so great. Why, for example, should only women who inherit a mutation in the *BRCA1* tumour suppressor be prone to breast cancer? Shridar Ganesan *et al.* have shown, in the November 1 issue of *Cell*, that BRCA1 might be involved in X inactivation. Perhaps this could explain this female-specific phenomenon.

BRCA1 was known to localize to the unpaired X chromosome in pachytene spermatocytes, and Ganesan *et al.* confirmed this by showing that BRCA1 colocalized with a component of the XY body. The XY body shows similarities to the inactive X (Xi) chromosome in female somatic cells — both are heterochromatic, silenced and are coated with the non-coding *XIST* RNA. So, does Xi also localize BRCA1?

Immunofluorescence of BRCA1 and fluorescent *in situ* hybridization (FISH) of *XIST*, carried out on female human cell lines, revealed that BRCA1 and *XIST* could colocalize to a nuclear structure — FISH of an X-chromosome probe confirmed that this was one of the X chromosomes. Chromatin immunoprecipitation analysis — using antibodies to BRCA1 or its binding partner BARD1 — followed by reverse-transcriptase PCR (RT-PCR) of *XIST* confirmed this interaction.

The next step was to investigate what happened in *BRCA1*-deficient cells. Frozen sections from sporadic breast and ovarian cancers were examined, and although the majority had nuclear BRCA1 and focal *XIST* (as opposed to diffuse) staining, those from *BRCA1*-deficient women did not. The HCC1937 human breast cancer cell line — which contains a germline mutation in one *BRCA1* allele and has lost the wild-type allele — also lacked focal *XIST* staining. This could be restored by ectopic expression of wild-type *BRCA1*, but not of cancer-associated *BRCA1* mutants. Similarly, RNAi of *BRCA1* in wild-type cells decreased focal staining of *XIST*.

So how does BRCA1 regulate *XIST* — through its localization, synthesis or stability? The authors carried out RT-PCR of *XIST* in HCC1937 cells that were transfected with either a vector control or with wild-type *BRCA1*, to distinguish between these alternatives. The levels of *XIST* RNA were equivalent in both transfected lines, indicating that BRCA1 can influence *XIST* localization, but not its synthesis or stability.

As *XIST* is required for X-chromosome inactivation, Ganesan *et al.* investigated whether loss of *BRCA1* influences the pattern of histone H3 methylation on lysine 9 (H3mK9), which is associated with transcriptional silencing. In female cells, there is a large amount of anti-H3mK9 antibody staining on Xi, but this is absent from HCC1937 cells. Similarly, H3mK9 immunofluorescence analysis on frozen sections of sporadic and BRCA1-deficient breast cancers indicates that BRCA1 is required for focal staining of H3mK9, and hence gene silencing.

But is loss of *BRCA1* expression sufficient to reactivate previously silenced genes? This was tested in a female mouse cell line in which one X chromosome carried a non-functional copy of *Xist* and the other, inactivated, X chromosome carried a silenced copy that was tagged with GFP. RNAi of *Brca1* resulted in the reactivation of *Xist*–GFP in a subset of these cells.

The loss of *BRCA1* in women might therefore reactivate genes that are normally silent on the Xi. The upregulation of a set of X-chromosomal genes in *BRCA1*-deficient ovarian cancers lends support to the importance of this phenomenon in promoting tumorigenesis, but the establishment of a firm link remains a future goal.

Emma Greenwood, Senior Editor,
Nature Reviews Cancer

References and links

ORIGINAL RESEARCH PAPER Ganesan, S. *et al.* *BRCA1* supports XIST RNA concentration on the inactive X chromosome. *Cell* **111**, 393–405 (2002)

WEB SITE

David Livingston's lab: http://www.hms.harvard.edu/dms/bbs/fac/livingston.html

IN BRIEF

FUNCTIONAL GENOMICS

Genome-wide DNA replication profile for *Drosophila melanogaster*: a link between transcription and replication timing.

Schübeler, D. *et al. Nature Genet.* **32**, 438–442 (2002)

It has been speculated that a link exists between the time at which a genomic region is replicated in S phase and its transcriptional activity, predominantly because early replication might allow DNA to be packaged into an 'open' chromatin conformation. A microarray-based approach for associating replication timing with gene expression found just such a correlation in *Drosophila*. This property might distinguish metazoans from lower eukaryotes, such as budding yeast, in which no correlation was found.

DEVELOPMENTAL BIOLOGY

Different regulation of T-box genes *Tbx4* and *Tbx5* during limb development and limb regulation.

Khan, P. *et al. Dev. Biol.* **250**, 383–392 (2002)

The limb identity gene *Tbx5* promotes limb initiation by interacting with *Wnt2b* and *Fgf10*.

Ng, J. K. *et al. Development* **129**, 5161–5170 (2002)

T-box genes *Tbx4* and *Tbx5* have been implicated in hindlimb and forelimb development, respectively. Khan *et al.* cloned *Tbx4* and *Tbx5* cDNAs from newt and showed that, unlike in higher vertebrates, both genes are expressed in the fore- and the hindlimbs. Interestingly, the authors found that, during limb regeneration, their expression is reminiscent of higher vertebrates' — *Tbx5* is preferentially upregulated in the forelimbs and *Tbx4* in the hindlimb — indicating that regeneration is not simply a reiteration of development. An additional role for *Tbx5* is revealed by Ng *et al.*, who used gain-of-function experiments in chick and zebrafish mutants and morpholino-based knock-down to show that *Tbx5*, together with *Wnt2a*, is also necessary and sufficient for limb outgrowth. The authors also show that *Tbx5* lies downstream of WNT and that it acts in a feedback loop with *Fgf10*.

TECHNOLOGY

Production of maternal-zygotic mutant zebrafish by germ-line replacement

Ciruna, B. *et al. Proc. Natl Acad. Sci. USA* 27 October 2002 (10.1073/pnas.222459999)

In zebrafish, and other organisms, maternally contributed RNAs and proteins can mask the effects of zygotic mutations. To overcome this, Ciruna *et al.* generated sterile fish by ablating primordial germ cells (PGCs) using morpholino oligos against the PGC transcript, *dead end*. PGCs from homozygous mutant donors were then transplanted into the sterile fish, where they repopulated the gonad and gave rise to maternal-zygotic mutants. This technique could be used to create large clutches of purely mutant embryos.

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