WEB WATCH

Getting to the core

- http://research.nhgri.nih.gov/bic/

The breast information core (BIC) is an international collaborative effort whose main goal is to facilitate the detection and characterization of breast cancer susceptibility genes, and, particularly, to share and coordinate non-published information between like-minded researchers. The group has a steering committee (headed by David Goldgar of the International Association of Cancer Research) and has a general meeting every year, which is apparently announced via a newsletter, although there is no link to one or to details of the meetings on the web site.

To view any information, you must register, and with that completed you can click onto the BIC database — the core of the site — and see the mutations data for *BRCA1* and *BRCA2*, which were last updated in August 2002. The databases can be searched by clicking on the exons themselves, or by using a form to search the genes. These data have also been summarized in several different ways for easier digestion — you can view graphs of missense or frameshift mutations, view a table of single-nucleotide substitutions, or a bar graph of insertions and deletions. Importantly, there is also a mutation submission form where you can submit your findings — these data are examined and edited by the steering committee to maintain the quality of the data.

So, although extra sections such as published laboratory methods (which consists of just two papers published in 1997) and a discussion forum (the link did not work) are not that useful at present, the core of the site is comprehensive and well worth a visit for *BRCA* researchers.

Ezzie Hutchinson

TUMOUR SUPPRESSORS

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 1 November 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 polymerase chain reaction (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, RNA interference (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.

DNA REPAIR

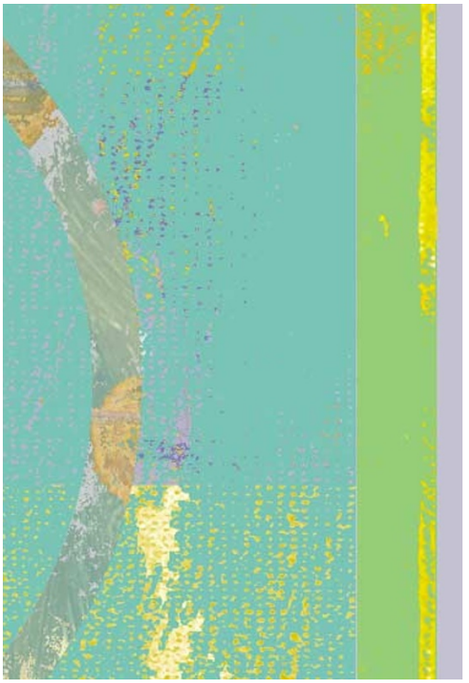
Molecular mimicry

Germline mutations in the *BRCA2* gene cause increased susceptibility to breast and ovarian cancers, so much work is being done to understand the cellular role of BRCA2. It is known to interact with — and modulate the function of — RAD51, a protein that is involved in recombinational DNA repair. But what does this interaction mean at a functional level?

Reporting in *Nature*, Tom Blundell, Ashok Venkitaraman and colleagues now reveal the structural basis for the BRCA2-dependent regulation of RAD51. They describe the 1.7-Å crystal structure of a complex between BRC repeat 4 (BRC4) of BRCA2 and the RecA-homology domain of RAD51, and show that the BRC repeat mimics a conserved motif found in RAD51, so enabling BRCA2 to control the activity of RAD51.

When mammalian cells are exposed to DNA damage, RAD51 oligomerizes on damaged DNA ends to form a nucleoprotein filament that is essential for subsequent steps in recombinational DNA repair. To work out how this happens, the authors compared the BRC4–RAD51 structure with that of the bacterial RAD51 homologue, RecA, which also forms a helical filament. They discovered that a conserved sequence motif in BRC4 structurally mimics a seven-amino-acid sequence that is found at the interface between subunits in the RecA filament. They then used this information to show that RAD51 oligomerizes through a similar motif. This motif is conserved in RecA-like molecules from bacteria to humans, highlighting a common structural mechanism for the formation of such nucleoprotein filaments.

BRCA2 binds to RAD51 through six of its eight so-called BRC repeats (BRC1–8). From the structure of the BRC4–RAD51 complex, the authors show that these BRC repeats mimic the structure of the natural RAD51 motif that forms the interface between RAD51 subunits in the nucleoprotein filament. So BRCA2 copies a structure in RAD51 to control the oligomerization state of RAD51.



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 H3mK9 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

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

Does this study tell us anything about BRCA2's role in cancer?

The authors show that several point mutations in *BRCA2*, which have previously been linked to cancer, impair the ability of BRCA2 to bind RAD51. RAD51 would therefore be unable to repair damaged DNA, which could explain the development of cancer. The importance of this interaction also means that the BRCA2–RAD51 interface could be a target for the development of small-molecule inhibitors as potential anticancer drugs.

Alison Mitchell
***Editor,* Nature Reviews Molecular Cell Biology**

References and links

ORIGINAL RESEARCH PAPER Pellegrini, L. *et al*. Insights into DNA recombination from the structure of a RAD51–BRCA2 complex. *Nature* 10 Nov 2002 (doi:10.1038/nature01230).

WEB SITE

Ashok Venkitaraman's lab: http://www.hutchison-mrc.cam.ac.uk/Venkitaraman.html

IN BRIEF

THERAPEUTICS

Synthetic small inhibiting RNAs: efficient tools to inactivate oncogenic mutations and restore p53 pathways.

Martinez, L. A. *et al. Proc. Natl Acad. Sci. USA* **99**, 14849–14854 (2002)

RNA interference is increasingly used to aid research by allowing the generation of functional knockouts. But can this technique be translated into the clinic to treat patients with cancer? Martinez *et al.* have constructed short interfering RNAs that can differentiate between wild-type and point-mutated p53. So, the mutant p53 — in tumours that express wild-type and mutant protein — could be selectively deleted, forming the basis of tailored therapy.

DIAGNOSTICS

Serum proteomic patterns for detection of prostate cancer.

Petricoin, E. F. *et al*. *J. Natl Cancer Inst.* **94**, 1576–1578 (2002)

At present, to confirm whether men with increased levels of prostate-specific antigen have prostate cancer or not, a biopsy sample must be taken. Petricoin *et al*. developed a bioinformatics algorithm that allowed discrimination between patients with prostate cancer and those with benign or no disease. The serum proteomic pattern correctly predicted 95% of patients known to have prostate cancer and 78% of patients with benign conditions.

IMMUNOTHERAPY

Dual-specific T cells combine proliferation and anti-tumor activity.

Kershaw, M. H., Westwood, J. A. & Hwu, P. *Nature Biotechnol*. **20**, 1221–1227 (2002)

Antitumour immunity requires T-cell activation, but tumour antigens are generally poor immunogens. To expand tumour-reactive T cells *in vivo*, Kershaw *et al*. generated dual-specific T cells that could respond not only to an immunogen, but could also recognize folate-binding protein (FBP) — an antigen associated with ovarian cancer. Mouse dual-specific T cells responded to both allogeneic antigen and FBP-expressing tumour cells *in vitro*, and expanded in response to immunization with allogeneic cells *in vivo*. Human dual-specific T cells were also generated.

THERAPEUTICS

Inhibitors of Ras/Raf-1 interaction identified by two-hybrid screening revert Ras-dependent transformation phenotypes in human cancer cells.

Kato-Stankiewicz, J. *et al. Proc. Natl Acad. Sci. USA* **99**, 14398–14403 (2002)

The signalling cascade that acts through RAS and RAF is activated in a significant number of tumours, so inhibiting their association is a viable therapeutic strategy. Small-molecule compounds that inhibit this interaction were identified using a two-hybrid approach. These prevented activation of the mitogen-activated protein kinase pathway and caused reversion of several *RAS*-transformed phenotypes, such as influencing morphology, invasiveness and anchorage-independent growth in a number of cell types.