WEB WATCH

Reach for the sky

- http://www.ncbi.nlm.nih.gov/sky/skyweb.cgi

A new database with the catchy title of 'NCI and NCBI Spectral Karyotyping *SKY* and Comparative Genomics Hybridization *CGH* Database' allows cancer researchers to share their SKY and CGH results with others. As a new site, it is, at present, lacking in data, but give it a few months and it could well be a useful information source.

SKY and CGH are fluorescent cytogenetic techniques that have revolutionized the visualization of chromosomal abnormalities. SKY facilitates the detection of chromosome breaks, translocations and rearrangements, as each chromosome is displayed in a different colour, and CGH identifies DNA gains and losses of whole chromosomes or parts of chromosomes. The home page includes a basic description of these, and provides links to a more detailed explanation.

So far, just two groups have submitted data, with a combined total of 27 cases. The results are stored under the name of the submitter, and are listed according to case number. Information on cancer type and site is available at this level, and links are provided to case details and the SKY/CGH data — which are available in a table and visually, as a cartoon. The cartoon chromosomes are themselves clickable and they link to a detailed chromosome map.

At present, the site is easy to navigate, but problems might come as the database grows. Advanced search facilities are under construction, but a 'quick search' (detailed in the help section) can be performed on several criteria. The line 'New database features (under construction)' on the home page indicates that work is ongoing; perhaps this will provide the finishing touches to a potentially valuable site.

Emma Greenwood

TUMOUR SUPPRESSORS

A dynamic duo

The *INK4A/ARF* locus codes for two overlapping transcripts, *INK4A* and *ARF*, both of which are frequently mutated in human cancer. Knocking out either *Arf* or both gene products predisposes mice to tumorigenesis, so what role does Ink4a (also known as p16) play? Two studies in the 7 September issue of *Nature* now address this problem, as Sharpless *et al.* and Krimpenfort *et al.* show that Ink4a is a *bona fide* tumour suppressor.

The two proteins are created by the use of different first exons and reading frames. Ink4a is a cyclin-dependent kinase inhibitor, which targets cyclin-D–Cdk4/6 and prevents phosphorylation of Rb; Arf, a known tumour suppressor, stabilizes p53 through its interaction with Mdm2. To investigate whether Ink4a also participates in tumorigenesis, both groups mutated the gene in mice, independently of *Arf*. Krimpenfort *et al.* introduced a stop codon in exon 2 to mimic a mutation that is frequently found in human tumours (*Ink4a*$^{*/*}$). This resulted in an unstable protein that could not be detected by immunoblotting. Sharpless *et al.* generated a knockout by deleting exon 1α (*Ink4a*$^{-/-}$). Interestingly, mouse embryo fibroblasts (MEFs) harbouring either of these mutations have the same growth rate as wild-type MEFs, show normal Rb phosphorylation patterns and can still respond to γ-irradiation and serum starvation by arresting in G1.

Ink4a accumulates with the onset of senescence in a process that depends on telomere shortening. However, both *Ink4a*$^{-/-}$ and *Ink4a*$^{*/*}$ MEFs undergo growth arrest in culture or when *RAS*, a potent oncogene, is overexpressed. In contrast, *Arf*$^{-/-}$ MEFs continue to grow at the same rate under these conditions, which indicates that Arf is the principal mediator of senescence. Despite this, *Ink4a*$^{-/-}$ cells still immortalize at a greater frequency than *Ink4a*$^{+/+}$ cells, and this does not always accompany loss of Arf or p53. Ink4a may therefore be able to facilitate escape from growth arrest.

When susceptibility to tumorigenesis was examined, some differences between the two groups' approaches emerged. Krimpenfort *et al.* showed no significant increase in the number of tumours when wild-type and *Ink4a*$^{*/*}$ mice were compared. Also, introduction of the *Ink4a*$^{*/*}$ mutation to Eμ-*Myc* mice — a well-established model of B-cell lymphoma — did not increase B-cell lymphomagenesis. However, when exons 2 and 3 of the second *Ink4a* allele were deleted — to generate a genotype frequently found in human tumours — there was a marked increase in tumour number. The remaining *Arf* allele was present in most of the tumours and gene silencing by methylation was not detected, so the increase in number was not due to loss of the second *Arf* allele.

One of the key uses for this mutant will be to model metastatic melanoma — the most predominant tumour type for humans with germ-line mutations at this locus. Application of DMBA, a known carcinogen, increased both the frequency of melanoma and the extent of metastasis.

Sharpless *et al.* showed that *Ink4a*$^{-/-}$ mice developed more tumours than wild-type and heterozygous mice; treatment with a variety of carcinogens further increased both malignancy and tumour type. As carcinogen-treated *Ink4a*$^{+/-}$ mice were more prone to tumours than wild-type mice, the authors investigated the status of the functional *Ink4a* allele in those tumours. Ink4a protein was not detected in any tumour; the *Ink4a/Arf* locus was not rearranged or deleted, but the gene could be epigentically silenced by promoter methylation — a mechanism of Ink4a loss also noted in many human tumours and an obvious target for therapy.

These papers provide a long-awaited answer to the conundrum of which protein is important in tumorigenesis — the answer being both. Questions for the future include which tumour suppressor is inactivated in which tumour type, and how this loss is brought about — through gene silencing or gene deletion.

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References and links

ORIGINAL RESEARCH PAPERS Krimpenfort, P. *et al*. Loss of *p16*^{Ink4a} confers susceptibility to metastatic melanoma in mice. *Nature* **413**, 83–86 (2001) | Sharpless, N. E. *et al.* Loss of p16^{Ink4a} with retention of p19^{ARF} predisposes mice to tumorigenesis. *Nature* **413**, 86–91 (2001)

FURTHER READING Sherr, C. J. Modulation of retinoblastoma and p53 by *INK4a/ARF*. *Nature Rev. Mol. Cell Biol.* **2**, 731–737 (2001)

WEB SITES

Anton Berns' lab: www.nki.nl/nkidep/h5/default.htm

Ron DePinho's lab: www.hms.harvard.edu/dms/bbs/fac/depinho.html