DNA REPAIR

# The guardian

Artemis is the Greek goddess that guards young children and small animals. The gene *Artemis* is therefore appropriately named, as it seems to be a genome guardian that protects children and mice from cancer. In the February issue of the *Journal of Clinical Investigation,* Moshous *et al.* associate mutations in *Artemis* with immunodeficiency and predisposition to lymphoma.

Artemis was initially identified as a factor involved in the non-homologous end-joining (NHEJ) phase of V(D)J recombination of T- and B-cell receptor genes. Disruption of this gene in mice resulted in a complete absence of T and B lymphocytes, as well as radiosensitivity.

Moshous *et al.* studied the effects of null and hypomorphic *Artemis* mutations in four patients from two families. These patients were born with radiosensitive severe combined immunodeficiency (RS-SCID), which is characterized by an absence of both mature B and T lymphocytes and gamma-ray sensitivity. After the appearance of lymphocytopaenia during infancy, the children developed severe infections, and two of the four developed B-cell lymphomas. Their peripheral blood lymphocytes were found to have chromosome instabilities — particularly in chromosomes where Ig and T-cell receptor genes were located. So, do these lymphocytes have defects in NHEJ DNA-repair machinery?

Moshous *et al.* found that T cells from these patients had a virtual absence of N nucleotide additions at the V(D)J junctions. N regions are non-templated nucleotides that are added to the 3′ ends of RAG-mediated double-strand breaks by the enzyme terminal deoxynucleotidyl transferase (TdT). N-region addition is known to require the NHEJ protein KU80, and these findings indicate that *Artemis* is also involved. Moshous *et al.* were able to complement the recombination

CANCER GENETICS

# Making sense of missense

*BRCA1* mutations have been linked to an increased risk of breast and ovarian cancer. Most of the highly penetrant alleles that have been identified encode truncated proteins, but missense alleles have also been recorded — their impact on cancer susceptibility is particularly difficult to assess because of low penetrance and the lack of adequate functional assays. In their paper, Fleming *et al.* describe an evolutionary approach to identify the missense alleles that are most likely to be associated with a disease phenotype.

The rationale behind this study is that mutations in functionally important amino acids are most likely to be associated with an increased risk of cancer. These key amino acids can be identified on the basis of their conservation in mammals or from evidence of recent positive selection in the human lineage. To find conserved *BRCA1* regions, the authors aligned GenBank sequences for exon 11 of *BRCA1* from 57 mammals. Considering a region of five amino acids in length at a time, regions were defined as conserved if at least 80% of the sites were fixed or conserved (that is, identical in all species or all residues sharing similar biochemical properties, respectively), including the first and last residues. Seven out of eight conserved regions were located in regions known to interact with other proteins. In addition, a conserved stretch of amino acids was identified in a region of unknown function that is also conserved in *BRCA1* homologues from *Xenopus* and chicken.

If site conservation is a good indicator of its functional importance, it would be predicted that missense mutations affecting fixed sites, or resulting in non-conservative substitutions at conservative sites, are most likely to be associated with a disease phenotype. In fact, 38 of the 139 documented missense alleles in exon 11 fall into one of these categories. The authors argue that these sites should be the priority for future studies, along with three missense mutations in residues that show signs of recent positive selection in human and primate lineages. Interestingly, these three amino acids lie in the region of BRCA1 that interacts with RAD51 — a protein that is involved in DNA double-strand-break repair.

The authors present a promising approach for prioritizing the study of missense mutations in *BRCA1*, as well as in other genes that are associated with heritable diseases. Indeed, the authors show that this method can be used to predict the β-globin amino-acid residues that, when mutated, are associated with various globin pathologies. The true test of this approach, however, will be to determine how many of the 41 missense mutations that have been highlighted by Fleming *et al.* are associated with an increased risk of breast and ovarian cancer.

*Catherine Baxter, Associate Editor,*
Nature Reviews Genetics

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deficiencies by re-expressing Artemis in the patients' fibroblasts, indicating that this gene alone is responsible for the DNA-repair defects.

This is some of the first solid evidence that NHEJ mutations are lymphomagenic in humans. Only one NHEJ deficiency has been found in humans — a hypomorphic mutation in ligase IV, which is associated with leukaemia and radiosensitivity — and the *Artemis* study is the first report of a human cancer that is associated with V(D)J defects. The authors suggest that other ill-defined immunodeficiency conditions associated with lymphoma should be investigated for defects in NHEJ factors.

*Kristine Novak*


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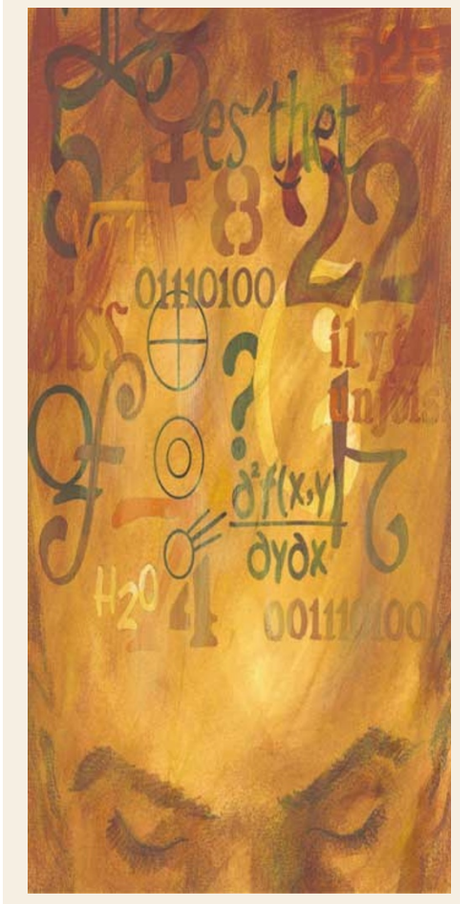

MELANOMA

# Sunburn effects dissected

Being sunburnt as a child is known to increase the risk of developing melanoma in later life, but what is happening at the molecular level to initiate tumour formation? Lynda Chin and colleagues now report in the *Proceedings of the National Academy of Sciences* that components of the retinoblastoma (RB) pathway are important targets of ultraviolet (UV)-light-induced mutagenesis.

To study the molecular impact of UV-light exposure, the authors used mouse models of melanoma that were driven by an activated *Hras* transgene (*Tyr-Ras*) and loss of the tumour suppressors Arf or Ink4a (both of which are encoded by *Cdkn2a*). ARF acts to stablize p53 and the cyclin-dependent kinase (CDK) inhibitor INK4A prevents CDK4/6-dependent phosphorylation of RB, so both lead to inhibition of cell-cycle progression. INK4A has been implicated in UV-light-induced mutagenesis because UV-light signature mutations of the gene have been found in human melanoma, although the occurrence of similar mutations in non-UV-light-induced cancers has raised questions regarding this connection.

*Tyr-Ras Arf*$^{-/-}$ mice that were treated with UV light developed more melanomas earlier than the transgenic animals that were not exposed to UV, although these mice did develop some tumours spontaneously. Having shown that UV light cooperates with HRAS and ARF to form tumours, the authors studied the effects of UV light on the INK4A–RB axis in this model. Functional loss of Ink4a was identified in 22% of tumours that arose in UV-light-treated *Tyr-Ras Arf*$^{-/-}$ mice — which is less than the 50% observed in mice not exposed to UV light. Instead, amplification and overexpression of *Cdk6* was seen in 46% of UV-light-treated mice and in none of the non-UV-light-treated mice. Alteration of *Cdk6* was not seen in the tumours with functional loss of Ink4a and vice versa — which is consistent with the fact that both genes cause the same phenotype. Loss of Rb itself was not seen in any tumour.

In contrast to ARF, the authors observed that loss of INK4A does not cooperate with UV light to accelerate melanoma development in *Tyr-Ras Ink4a*$^{-/-}$ mice. This was a surprising finding given the widely held assumption that UV light has a broad mutagenic action. Instead, this result indicated that UV light exclusively targets the RB pathway for inactivation. So, the authors hypothesize that UV-light exposure increases melanoma risk by inactivating the RB pathway — either by means of Ink4a loss or Cdk6 amplification.

Results of this study indicate that analysis of INK4A, CDK6 and other RB-pathway components in people with a history of sunburn might be useful for the early detection of individuals who have a particularly high risk of developing melanoma. As there are no effective therapies for advanced melanoma, catching these cancers at an early stage would represent an important clinical advance.

*Ezzie Hutchinson*


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**WEB SITE**

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