P53 REGULATION

3 out of 4 ain't bad

According to Meatloaf, "2 out of 3 ain't bad", but when it comes to p53 regulation, homeodomain-interacting protein kinase-2 (HIPK2) can improve on these odds. Two papers in the January issue of *Nature Cell Biology* describe how it is involved in three out of the four ways in which p53 is regulated.

Thomas G. Hofmann *et al.* had previously cloned human HIPK2, but Gabriella D'Orazi *et al.* isolated HIPK2 in a yeast two-hybrid screen, using a p53 mutant that lacked the transactivation domain as the bait. This provided the first insight into its function.

The HIPK2–p53 interaction was confirmed *in vitro* and *in vivo*. Hofmann *et al.* showed it to be independent of HIPK2's kinase activity; a point mutation (K221A) renders the kinase inactive, yet it can still bind p53. HIPK2 and p53 also co-localize in nuclear structures known as nuclear bodies, which is facilitated by PML3 (see picture). CREB-binding protein (CBP), which acetylates and activates p53, also localizes in nuclear bodies, indicating that they could be structures in which p53 is modified.

Could HIPK2 regulate p53's function as a transcriptional activator? Hofmann *et al.* transfected SAOS-2 cells with a luciferase reporter controlled by the promoter of the *CDKN1A* gene — which encodes WAF1, a cyclin-dependent kinase inhibitor and known p53 target. They showed that HIPK2 could induce luciferase expression in a p53-dependent manner, but only when its kinase activity was intact. D'Orazi *et al.* used a similar system, this time in H1299 cells, and found that although HIPK2 increased the luciferase expression from the *MDM2* promoter in a dose-dependent manner, it had no effect on that driven by the *CDKN1A* promoter. HIPK2 therefore seems to be both cell-type and promoter specific.

So, does HIPK2 directly phosphorylate p53 to enhance p53-dependent transcription? HIPK2 could phosphorylate p53 on Ser46 *in vitro* and *in vivo*, and Hofmann *et al.* showed that a p53 mutant in which Ser46 was replaced with alanine (p53-S46A) was much less able than wild-type p53 to induce expression from the *CDKN1A* promoter in response to HIPK2.

p53's Ser46 residue is phosphorylated in response to ultraviolet (UV) irradiation, so does UV irradiation activate HIPK2? D'Orazi *et al.* showed that, following UV irradiation, HIPK2's expression levels increased, it co-precipitated with p53 and it could phosphorylate p53. Hofmann *et al.* also showed that UV irradiation induced co-localization of HIPK2, p53 and CBP in nuclear bodies. In fact, HIPK2 and CBP were shown to interact, and acetylation-specific antibodies indicated that expression of *HIPK2*, but not the kinase-deficient mutant, induces acetylation of p53's Lys373 and Lys382 residues. Insight into this mechanism was provided when the p53-S46A mutant was found to remain unacetylated when HIPK2 and CBP were co-expressed in H1299 cells. So, UV-irradiation-induced HIPK2-mediated phosphorylation of Ser46 is required for acetylation, and this further increases the transactivation activity of p53.

So what is the consequence of HIPK2 activation and the corresponding increase in p53 activity — apoptosis or cell-cycle arrest? D'Orazi *et al.* showed that the addition of antisense oligonucleotides to HIPK2 led to a fivefold decrease in apoptosis, as quantitated by the TUNEL assay. Both groups also showed that expression of *HIPK2* suppresses growth in a colony formation assay, and D'Orazi *et al.* went on to attribute this to apoptosis: cells stained positive for TUNEL; a hypodiploid peak was present when cells were analysed for DNA content; and there was an increase in expression of the apoptotic genes *BAX* and *PIG3*. Hofmann *et al.* confirmed that apoptosis was enhanced, but also showed that cells arrested in G1, owing to an increase in WAF1 levels.

So, this newly identified p53 regulator is involved in localization, phosphorylation and acetylation of p53 in response to UV irradiation. Indeed, the only mechanism of p53 control that it doesn't seem to affect is degradation. Perhaps HIPK2, like so many other regulators of p53, will turn out to be a tumour suppressor in its own right.

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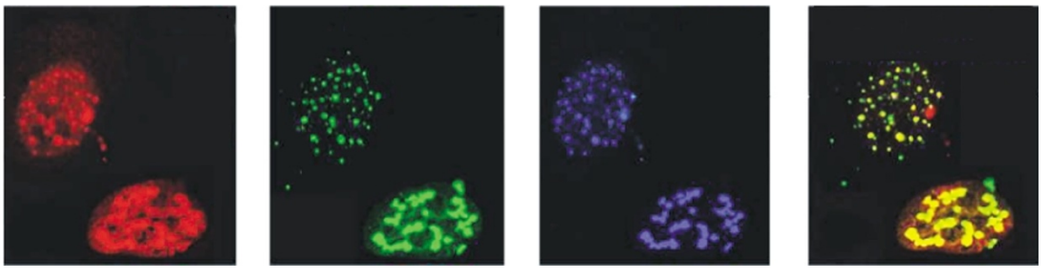
References and links

ORIGINAL RESEARCH PAPERS Hofmann, T. G. *et al.* Regulation of p53 activity by its interaction with homeodomain-interacting protein kinase-2. *Nature Cell Biol.* **4**, 1–12 (2002) | D'Orazi, G. *et al.* Homeodomain-interacting protein kinase-2 phosphorylates p53 at Ser 46 and mediates apoptosis. *Nature Cell Biol.* **4**, 13–19 (2002)



Expression of PML3 (blue) facilitates localization of p53 (red) and HIPK2 (green) within nuclear bodies. The final panel is a merged image of p53 and HIPK2. Reproduced with permission © (2002) Macmillan Magazines Ltd.

WEB WATCH

Breast is best

- http://mammary.nih.gov/index.html

Ever wondered where to go for mammary gland information? Now you need look no further than the 'Biology of the Mammary Gland' web site. It's divided into seven sections, and is easy to search and navigate; however, it could do with an update, as 1999 seems to be the most recent entry.

One of the most useful areas of the site is 'experimental models'. It is split into three parts: transgenic mice, deletion mice and natural mutants, so provides information on many mouse models that are relevant to breast cancer research. The 'reviews' section contains a series of articles, ranging from development and gene expression to apoptosis and tumorigenesis, and is also a good source of information.

The 'tools and technologies' section would certainly help those new to the field, as would the 'histology atlas' — a catalogue of images that describe development in normal, knockout and transgenic mice. It even includes a section on how to diagnose breast cancer. This has potential, but is limited by its gaps, as some of the links no longer work. These two sections are complemented by the 'methodology online CD-ROM', which also features histology.

Although most of the site is filled with information that is directly relevant to research, it is not lacking history or humour. The 'art and history' section within 'about us' includes pictures such as 'Sacred milk', and even features a Beatles song!

So, although currently useful, parts of the site will become increasingly less so, unless it is updated. Let's hope that the wealth of new information on mammary gland biology is soon included.

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