BRIEF COMMUNICATIONS

Policing of oncogene activity by p53

Oncogenes, rather than DNA damage, may provide the key signal to p53 to trigger tumour suppression.

The tumour-suppressor protein p53 provides the most important genetic defence against cancer[1] and is activated in response to DNA damage and to oncogenic signalling, both of which occur almost universally in malignant tumours. But the relative contribution of these two pathways in inducing p53-dependent protection against cancer is unclear. Here we show that p53-dependent protection against cancer is lost in mice that have been genetically manipulated so that their p53 is activated in response to DNA damage but not to oncogenic signalling. We conclude that oncogenic signalling is the critical event that elicits p53-dependent protection and that the DNA-damage stimulus is less important.

DNA damage and oncogenic signalling are communicated to p53 through separate routes, which are, respectively, a p53-phosphorylation cascade that involves the ATM/Chk2/ATR/Chk1 series of kinases, and a p53-stabilization pathway that requires the tumour-suppressor protein ARF and the ubiquitin-ligase MDM2 (ref. 1).

To investigate the role of oncogenic signalling in p53-mediated protection against cancer, we used mice with two genetically engineered traits: one had no *ARF* allele (ARF^{null} mice)[2] and the other had a 'super' *p53* allele[3] ($p53^{super}$ mice; these mice carry a single additional transgenic copy of the intact *p53* gene, which behaves in the same way as endogenous *p53*). Compared with wild-type mice ($p53^{wt}$ mice), which have just two copies of *p53*, the $p53^{super}$ mice have additional protection against cancer development[3]. This experimental system is therefore well suited for quantifying p53-dependent protection against cancer. (See supplementary information for methods.)

Before analysing their susceptibility to cancer, we confirmed that ARF^{null} mice respond normally to DNA damage[4,5] by showing that apoptosis of their thymocytes after irradiation was unaffected (Fig. 1a). We found that mice with the $p53^{super}$ allele showed the same enhancement of apoptosis irrespective of whether ARF was present or absent (Fig.1a). However, ARF^{null} cells were unable to respond effectively to oncogenic signalling[6–8] and underwent neoplastic transformation by oncogenes *in vitro*, irrespective of the presence or absence of the $p53^{super}$ allele (Fig. 1b).

As p53 responds normally to DNA damage in the absence of ARF, we reasoned that $p53^{super}$ might provide some protection against tumour development *in vivo*, even without the ability to detect oncogenic signalling. However, we found that $p53^{super}/ARF^{null}$ mice succumbed to spontaneous tumours at the same rate as $p53^{wt}/ARF^{null}$ mice (Fig. 1c), producing the same profile of sarcomas, lymphomas and histiocytic sarcomas (results not shown).

We also treated $p53^{super}/ARF^{null}$ and $p53^{wt}/ARF^{null}$ mice with the DNA-damaging agent 3-methyl cholanthrene. This agent produces DNA adducts and results in fibrosarcomas carrying oncogenic mutations in *ras* genes (see supplementary information). This carcinogenic protocol is highly sensitive to the functionality of p53, as indicated by the greater resistance to the agent of $p53^{super}$ mice compared with $p53^{wt}$ mice[3]. As with the spontaneous tumours, the extra gene dose of *p53* became irrelevant in the absence of ARF (Fig. 1d).

Together, our results indicate that the cancer-protective activity of p53 is abolished in the absence of ARF. We conclude that oncogenic signalling is critical for triggering protection by p53, whereas activation of p53 as a result of DNA damage has a lesser impact on the ultimate development of tumours. Although there are differences in these pathways in mice and humans, our findings may also explain the high incidence of ARF loss in human cancers[9], as well as the low incidence of mutations in the kinase enzymes of the p53-phosphorylation cascade[10] that is induced by DNA damage.

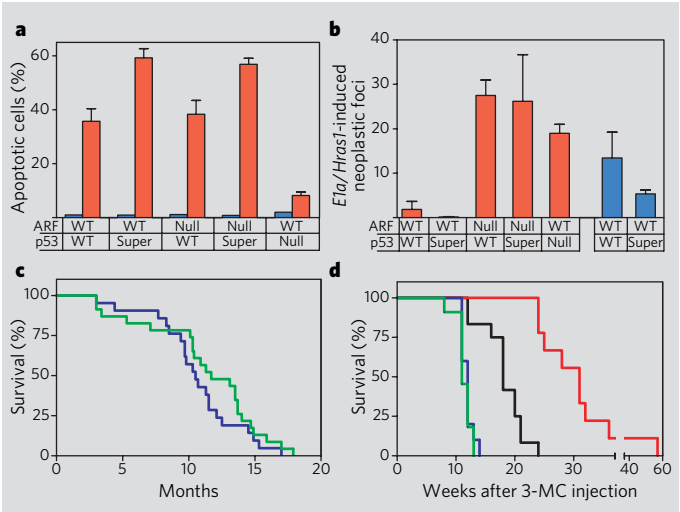

Figure 1 | ARF is necessary for tumour suppression by p53. a, The p53-dependent DNA-damage response *in vivo* does not depend on ARF. Mice ($n = 3$ per genotype) were irradiated (10 Gy) and the percentage of apoptotic thymocytes was determined 3 h later. Blue bars, non-irradiated controls; red bars, cells from irradiated mice. **b**, ARF is essential for the defensive response to oncogenic signals. Primary embryonic fibroblasts (from $n = 2$ embryos per genotype) were retrovirally transduced with *E1a* and oncogenic *Hras1* and plated as indicated, and the number of resulting neoplastic foci was scored. Red bars, 2,000 cells; blue bars, 50,000 cells. WT, wild type. **c**, Lifespans of $ARF^{null}/p53^{wt}$ and $ARF^{null}/p53^{super}$ mice ($n = 21$ (blue) and $n = 23$ (green) per genotype, respectively) were not significantly different (log rank test, $P = 0.26$). **d**, Mice of genotype $ARF^{wt}/p53^{wt}$ ($n = 12$; black), $ARF^{wt}/p53^{super}$ ($n = 9$; red), $ARF^{null}/p53^{wt}$ ($n = 10$; blue) or $ARF^{null}/p53^{super}$ ($n = 11$; green) were treated with the DNA-damaging agent 3-methyl cholanthrene (3-MC) and monitored for the development of fibrosarcomas. The protection against tumour development provided by the $p53^{super}$ allele in the presence of ARF disappears in the absence of ARF; (see also supplementary information).

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