

**In the right place at the right time – analysis of p53 Serine 312 phosphorylation
in vivo**

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As a master sensor of stress, p53 is extensively regulated by post-translational modifications in response to stress signals. Phosphorylation is the most studied post-translation modifications of p53. p53 is phosphorylated at several serines, usually at the N- or C-terminus, and lysine residues within the C-terminus can also be modified^{1, 2}. However, these residues are infrequently mutated in tumours (<http://www-p53.iarc.fr>), and consistent with this, mice carrying mutations that prevent the modification of these amino acids possess subtle phenotypes²⁻⁴. Some (e.g. mice with mutations to Ser18 and Ser23) develop spontaneous tumours, frequently lymphomas, although with a late onset (1-2 years). In contrast p53^{-/-} mice, or mice with tumour-derived mutations, generally survive less than one year. Mice with C-terminal lysine to arginine mutations show some defects at a cellular level, but do not develop spontaneous tumours^{3,4}.

The failure to observe clear phenotypes in post-translational modification defective p53 knock in mice is because we still know little about how defined stimuli regulate p53 via specific post-translational modifications on specific sites. Thus, how the multi-layered and complex regulation of p53 activity by post-translational modification affects its tumour suppressive function can only be revealed if the mouse is subject to the right stress signal at the right time. This is supported by two recent studies of p53 Ser312Ala knock in mice.

Recently, both ourselves and Lee et al. have independently published papers describing mice carrying a serine to alanine mutation at codon 312, equivalent to human serine 315^{5, 6}. This residue is phosphorylated by kinases such as cdk2, Aurora A kinase and GSK3 β ². Both studies found that p53^{312A/A} mice do not develop spontaneous neoplasms and have a lifespan similar to wild type mice.

However when p53 Ser312Ala mice were exposed to a DNA damaging stimulus, opposing results were obtained. Both studies exposed the mice to a single 4Gy dose of radiation and monitored the mice for tumour development. Lee et al. found that the p53^{S312A/S312A} were no more susceptible to tumour development than the wild type controls, whereas in Slee et al's study the homozygous mice were significantly more tumour-prone than both heterozygous and wild type mice. In particular, these mice were predisposed to thymic lymphomas and liver tumours. The lymphomas developed between 25 and 50 weeks of age, with the liver tumours arising between 70 and 90 weeks, a time frame comparable with tumour onset in p53^{+/-} mice⁷. This, combined with reduced elevation in p21 expression in cells and tissues derived from these mice and partial rescue of the embryonic lethality caused by the absence of Mdm4, suggests that p53^{312A/A} is less active than wild type p53⁵.

An explanation for these contrasting results may lie in the timing of the X-ray exposure. The mice used by Lee et al. were exposed at 5 weeks, compared with 3 weeks in Slee's study. A substantial proportion of the p53^{312A/A} mice in Slee's study developed thymic lymphomas, a form of tumour that commonly develops in mice that lack full p53 function^{7, 8}. The mouse thymus continues to develop for 3 to 4 weeks after birth, and the rate of T-cell production by the thymus is greatest before puberty. After puberty, the thymus shrinks, production of new T cells in adults is lower, and removal of the thymus after puberty does not result in any loss of T-cell function. As puberty in mice occurs around 25-30 days after birth, the 3 week old mice irradiated in our study were immature, whereas the 5 week old mice used by Lee et al. would have been pubescent. We therefore speculate that phosphorylation of p53 on Ser312 plays a more important role in influencing p53's tumour suppressive function in pre-pubescent mice than in those that are post-pubescent. It is important to note that in p53 null, p53^{+/-} or tumour-derived p53 mutant mice the defect in p53 is profound, so tumours arise either spontaneously or irrespective of the timing of radiation exposure. Timing becomes important with a mutation such as Ser312Ala, which has a subtle impact on p53's tumour suppressive function.

As many post-translational modifications arise following DNA damage, it is possible that exposing mice carrying mutated modified residues to a

stimulus such as radiation will reveal a more dramatic phenotype, like the one observed in the p53^{312A/A} mice. For example, mice carrying a Ser389Ala mutation at p53's C-terminus are more susceptible to skin tumours following UV exposure, and bladder tumours after dosage with 2-acetylaminofluorene, although they do not develop tumours without these stimuli. In contrast, their response to ionising radiation is no different to wild type^{9, 10}. Therefore, this p53 modification responds to specific stimuli only in certain tissues. As yet, no other phosphorylation or acetylation mutants have been recorded as having been exposed to p53-activating stimuli: to do so could provide valuable insights into the biology of p53.

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