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P53 functional studies with Hupki (human p53 knock-in) mouse model

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p53 plays important roles in regulating various cell responses to DNA damage and other forms of stress, including cell cycle arrest, apoptosis, DNA repair and inhibition of angiogenesis and metastasis. Mutations in the p53 gene are found in the majority of human tumors, and are believed to be one of the key steps in carcinogenesis. Most of the mutations in humans have been found in the sequence encoding the p53 DNA binding domain (DBD). The DNA DBD sequence is 85% homologous at the nucleotide level in mice and humans. Despite this high homology, the use of mice to compare mutation spectra or to perform in vivo functional studies on human p53 variants has its limitations. Some of these may be overcome by using the human p53 knock-in (Hupki) mouse, in which the polyproline domain (PPD) and DBD of the murine p53 gene were replaced by the human homolog. The Hupki strain appears to be phenotypically "wild-type" with respect to development and tumor susceptibility. Furthermore, Hupki p53 shows typical p53 wild-type responses to DNA damage and other apoptotic stimuli.

To characterize an *in vitro* system with Hupki cells for generating human p53 mutation spectra, p53 functional studies were performed with cell lines immortalized from Hupki 13.5 day embryonic fibroblasts (HUFs). The immortalized cell lines that harbor typical p53 mutations of human tumors were compared with those that retained wild type p53 sequences. First, transcriptional induction following γ-irradiation of p53 target genes p21/WAF1, Mdm2 and Puma was determined by real time quantitative RT-PCR in a set of 21 HUF cell lines, 12 with mutant p53 and 9 with wild-type p53 PPD and DBD. Inducibility of all three genes showed a positive correlation with retention of functional wild type p53. Second, the genetic

alterations responsible for immortalization of Hupki cells that retain wild-type p53 sequences were investigated. It is known that fibroblasts from normal mice with murine p53 become immortalized either by p53 mutation, or by inactivation of another key regulator and tumor suppressor, p19ARF. p19ARF expression, which was determined by real time quantitative RT-PCR and immunoblot analysis, was upregulated in all 12 Hupki cell lines with mutant p53, while it was downregulated or undetectable in the p53 wild-type HUFs. In keeping with observations in mouse tumors, p19ARF was inactivated by deletion in 3 of 9 p53 wild-type HUFs, whereas none of the wild-type p53 lines had suffered p19ARF coding sequence mutations. As in normal tissue, but in contrast to results with tumor samples reported by others, no methylated CpG islands in the p19ARF promoter of HUFs were found that correlated with low p19ARF expression. This is a clear difference between the HUF immortalization pathway and the genetic steps leading to tumorgenesis. Further elucidation of the various pathways to immortalization of HUFs will be useful in refining the strategies and protocols currently followed to study spontaneously arising and mutagen-induced p53 mutations in Hupki cells.

To investigate the behavior of p53-inducible genes in HUFs in response to UVC-irradiation, and to search for novel candidate p53 targets, gene expression profiling was performed in 3 cell lines, 42B1 (p53 wild-type HUF), 32B1 (p53 mutant HUF, S127F) and AP43a (p53 null MEF) with DNA oligonucleotide microarrays (Affymetrix murine U74A, 12,000 transcripts). Expression changes of 3 known p53 target genes (p21/WAF1, Mdm2 and Apaf1) and 4 genes we identified as potential candidates (Dnmt1, CCL2, Eph1/mEH and Cryz) were confirmed by real time quantitative RT-PCR. One of the candidates, CCL2, was investigated further in order to determine whether its transcription was indeed directly regulated by p53. Nine of ten putative p53 consensus binding sites at this locus, which were identified by the p53MH algorithm, showed no response to p53 by reporter gene assay. Experiments to silence p53 with the specific chemical inhibitor pififthrin-α or by siRNA failed to support the existence of a direct correlation activity between p53 and CCL2 expression. Another candidate we considered, Dnmt1, was recently showed to be a direct p53 target gene by another group. The strategy of identifying p53 inducible genes by comparing expression

profiles in cell lines differing in p53 status has the limitation that cells may be immortalized by various mechanisms in vitro and in addition, acquire numerous other heterogeneous genetic changes during in vitro culturing, confounding simple comparisons.