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article

A teratologic suppressor role for *p53* in benzo[a]pyrene-treated transgenic *p53*-deficient mice

Dawn - Mutation

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DNA damage may mediate birth defects caused by many drugs and environmental chemicals, therefore *p53*, a tumour suppressor gene that facilitates DNA repair, may be critically embryoprotective. We have studied the effects of the environmental teratogen, benzo[a]pyrene, on pregnant heterozygous p53-deficient mice. Such mice exhibited between 2- to 4-fold higher embryotoxicity and teratogenicity than normal *p53*-controls. Fetal resorptions reflecting *in utero* death were genotyped using the polymerase chain reaction and found to be increased 2.6-fold and 3.6-fold respectively with heterozygous and homozygous p53-deficient embryos. These results provide the first direct evidence that *p53* may be an important teratological suppressor gene which protects the embryo from DNA-damaging chemicals and developmental oxidative stress.

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Teratogenesis, like cancer, may be initiated by DNA damage caused by radiation and chemicals[1]. One of the cell's important defences against the incorporation of DNA damage into daughter cells is the p53 protein, which is postulated to exert its protective effect via several mechanisms. In response to DNA damage, p53 can inhibit DNA replication by triggering a growth arrest in G1 (ref. 2) or initiate apoptosis[3–5]. The importance of functional p53 in relation to cancer has been demonstrated by the observations that the *p53* gene is mutated in over 50% of human cancers[6], and that individuals with the Li-Fraumeni syndrome, who inherit *p53* germline mutations, are extremely susceptible to cancer development[7,8]. Furthermore, mice deficient in the *p53* gene at one or both alleles also develop cancers at an early age[9,10].

DNA damage is thought to initiate the teratogenicity caused by numerous drugs and environmental chemicals, collectively termed xenobiotics[1]. Elevated levels of p53 protein have been observed in cell culture after exposure to DNA-damaging agents[2,11], and have been correlated with the triggering of both cellular growth arrest[2,12] and apoptosis[11]. Since p53 mRNA and protein are expressed in mouse embryos during the period of organogenesis[13,14], we postulated that *p53* might be an important teratological suppressor gene, with *p53* deficiencies enhancing susceptibility to chemical teratogenesis and *in utero* death (Fig. 1).

To test this hypothesis, we chose benzo[a]pyrene, representative of a multitude of polycyclic aromatic hydrocarbons found widely in the environment, and a classic DNA-damaging carcinogen[15] and teratogen[16]. Although the parent compound is relatively non-toxic, benzo[a]pyrene is bioactivated *in vivo* by cytochromes P450 and peroxidases to highly toxic electrophilic and free radical reactive intermediates which, if not detoxified, irreversibly damage DNA by covalent binding[17] or oxidation[18] (Fig. 1).

Cytochromes p450 in *p53*-deficient mice

As the *p53*-deficient (TSG-*p53*) mouse is a new transgenic strain that has not been characterized for xenobiotic metabolizing activities, our first concern was to determine whether CYP1A1, the major P450 isoform that bioactivates benzo[a]pyrene, was inducible in this strain. This question is important because CYP1A1 is a non-constitutive P450, and enzyme induction is necessary for achieving substantial bioactivating activity[19]. Since TSG-*p53* mice were derived largely from the C57BL/6 strain[9], which is genetically CYP1A1-inducible[20,21], a similar pattern of substantial CYP1A1 inducibility was anticipated for the TSG-*p53* strain.

To induce CYP1A1, pretreatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was chosen in part because of its high potency and broad efficacy as a CYP1A1 inducer among both inducible and resistant murine strains[20]. Perhaps more critically, TCDD, unlike benzo[a]pyrene, is not a substrate for CYP1A1 (ref. 22), and hence is not bioactivated to a toxic reactive intermediate. Accordingly, while TCDD itself in high doses is teratogenic, these effects are initiated by the same process by which it induces CYP1A1; namely, binding with high affinity to the aromatic hydrocarbon (Ah) receptor[20], rather than via a toxic reactive intermediate. Not only are the teratological anomalies initiated by TCDD[23] different from those initiated by benzo[a]pyrene[24], but also the dose of TCDD necessary to induce CYP1A1