Genetic interaction between *HAP1/REF-1* and *p53*

Mice defective in the *XPC* gene required for nucleotide excision repair (NER) of DNA are highly predisposed to skin cancer after exposure to UVB radiation (Fig. 1; refs 1–3). The *p53* gene (also known as *TP53*) has also been implicated in the pathogenesis of cancers in mammals[4]. We previously reported that the onset of skin cancer is accelerated in $XPC^{-/-}$ animals with one *p53* allele deleted (Fig. 1; ref. 3). We now show that $XPC^{-/-}p53^{-/-}$ mice are even more cancer prone than $XPC^{-/-}p53^{+/-}$ animals (Fig. 1). Hence, the absence of p53 protein provides a synergistic effect in promoting skin cancer in *XPC* mutant mice.

To investigate the role of base excision repair in environmental carcinogenesis, we have focused on the *HAP1* gene, which encodes the major apurinic/apyrimidinic (AP) endonuclease in mammalian cells[5]. HAP1 protein was independently discovered as a redox protein, which is required for the activation of oxidized AP-1 transcription factor[5,6]. Hence, *HAP1* is also referred to as *REF1* (*re*dox *f*unction; refs 5,6). Homozygous deletion of mouse *HAP1/REF1* results in embryonic lethality (L.B.M., D.L.C., R.E.H. & E.C.F., unpublished observations; ref. 7). Heterozygous *HAP1* mutants are viable, however, and grow normally.

We introduced the *HAP1* heterozygous state into animals carrying mutations in the *XPC* or *p53* gene and exposed them to UV radiation as described[3]. $XPC^{-/-}p53^{+/+}HAP1^{+/-}$ mice manifested accelerated skin cancer compared to $XPC^{-/-}p53^{+/+}HAP1^{+/+}$ animals (Fig. 1). This effect was not observed in $XPC^{+/+}p53^{+/+}HAP1^{+/-}$ animals, which showed a skin-cancer rate indistinguishable from that in wild-type animals (Fig. 1). Significantly, the kinetics of cancer induction in $XPC^{-/-}$ mice that are additionally heterozygous for both *p53* and *HAP1* was indistinguishable from that in $XPC^{-/-}$ animals heterozygous for just *p53* (Fig. 1). Furthermore, the kinetics of induction of skin cancer in $XPC^{-/-}p53^{-/-}$ double mutants was not enhanced by the *HAP1* heterozygous state (Fig. 1). $XPC^{+/+}p53^{+/-}HAP1^{+/-}$ mice showed no increased cancer predisposition (Fig. 1). These observations suggest that inactivation of *HAP1* or *p53* synergizes with defective NER by the same mechanism(s).

Recent biochemical studies have provided evidence that HAP1 is a potent activator of p53, operating by both redox-dependent and redox-independent mechanisms[8]. Our experiments provide distinct genetic evidence that activation of p53 protein is *HAP1* dependent. Hence, in $XPC^{-/-}$ animals with a deletion of one *HAP1* allele, loss of the second allele in pre-neoplastic or neoplastic cells may render cells phenotypically *p53* null. The observation that $XPC^{-/-}$ mice heterozygous for *HAP1* are not quite as cancer prone as $XPC^{-/-}$ mice heterozygous for *p53* suggests that *HAP1* may be less susceptible than *p53* to mutations that inactivate the function of p53 protein.

Because the redox function of HAP1 is apparently required for activation of oxidized AP-1 and possibly other transcription factors[5,6], the possibility that defects in these functions might somehow account for the results observed cannot be formally discounted. Experiments to demonstrate and map the location of mutations in the second *HAP1* allele in skin tumours from $XPC^{-/-}p53^{+/+}HAP1^{+/-}$ animals are in progress.

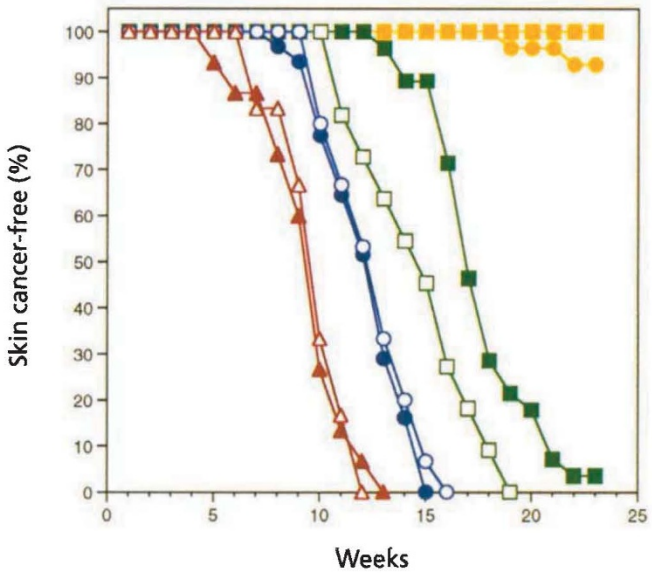

Fig. 1 Kinetics of radiation-induced skin cancer in mice. Animals were exposed to daily UVB radiation on the shaved dorsal skin as previously described[3]. Mice were examined and scored for skin cancers at least once a week, and all tumours were confirmed by histological examination. The curves represent 18 $XPC^{+/+}p53^{+/+}HAP1^{+/+}$, 5 $XPC^{+/+}p53^{+/+}HAP1^{+/-}$ and 4 $XPC^{+/+}p53^{+/-}HAP^{+/-}$ animals (yellow squares); 28 $XPC^{+/+}p53^{+/-}HAP1^{+/+}$ animals (yellow circles); 28 $XPC^{-/-}p53^{+/+}HAP1^{+/+}$ animals (green squares); 12 $XPC^{-/-}p53^{+/+}HAP1^{+/-}$ animals (green open squares); 30 $XPC^{-/-}p53^{+/-}HAP1^{+/+}$ animals (blue closed circles); 16 $XPC^{-/-}p53^{+/-}HAP1^{+/-}$ animals (blue open circles); 15 $XPC^{-/-}p53^{-/-}HAP1^{+/+}$ animals (red closed triangles); 6 $XPC^{-/-}p53^{-/-}HAP1^{+/-}$ animals (red open triangles).

Acknowledgements

The contributions of L.B.M. and D.L.C. are considered equal. Studies were supported by a research grant from the USPHS (E.C.F.) and by fellowships from the ACS (D.L.C.) and Friends of the Center for Human Nutrition, University of Texas Southwestern Medical Center (L.B.M.).

Lisiane B. Meira[1], David L. Cheo[1], Robert E. Hammer[2], Dennis K. Burns[1], Antonio Reis[1] & Errol C. Friedberg[1]

[1]Laboratory of Molecular Pathology, Department of Pathology, and [2]Department of Biochemistry and Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, Texas 75237 USA. Correspondence should be addressed to E.C.F. e-mail: friedberg.errol@pathology.swmed.edu

1. Cheo, D.L. *et al. Mutat. Res.* **374**, 1–9 (1997).
2. Sands, A.T., Abuin, A., Sanchez, A., Conti, J.C. & Bradley, A. *Nature* **377**, 162–165 (1995).
3. Cheo, D.L. *et al. Curr. Biol.* **6**, 1691–1694 (1996).
4. Levine, A. *Cell* **88**, 323–331 (1997).
5. Barzilay, G. Walker, L.J., Rothwell, D.G. & Hickson, I.D. *Br. J. Cancer* **4** (Suppl. XXVII), S145–S150 (1996).
6. Xanthoudakis, S., Miao, G., Wang, F., Pan, Y-C.E. & Curran, T. *EMBO J.* **11**, 3323–3335 (1992).
7. Xanthoudakis, S., Smeyne, R.J., Wallace, J.D. & Curran, T. *Proc. Natl. Acad. Sci. USA* **93**, 8919–8923 (1996).
8. Jayaraman, L. *et al. Genes Dev.* **11**, 558–570 (1997).