Can p53 Status Resolve Paradoxes Between Human and Non-Human Retinoblastoma Models?

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The cloning of the retinoblastoma gene (RB) stands as a landmark in modern cancer research as it was the first human cancer susceptibility gene isolated (1), it immediately validated the two-hit hypothesis for tumorigenesis (2), and it has served the scientific community for the past 11 years as a paradigm for the concept of a tumor suppressor gene. In addition, subsequent work on the functional properties of the RB product has provided clues that begin to link the biology of DNA tumor viruses, the eukaryotic cell cycle, the regulation of RNA polymerases, and pathways of mammalian carcinogenesis (3). Despite these remarkable advances, however, several unanswered questions remain. In particular, a fundamental paradox comes to mind when considering the study of Schlamp et al. (4), which is reported in this issue of the Journal. Why are humans the only known animal species that spontaneously develop retinoblastoma tumors? A dramatic confirmation of this surprising observation is the demonstration that RB(+/-) gene knockout mice (the counterpart to individuals with human familial retinoblastoma) never develop retinal tumors, although these heterozygous mice eventually succumb to pituitary tumors with a high penetrance (5,6). While a satisfactory (i.e., reductionist) model that can incorporate these findings is still lacking, experimental data over the past two decades have suggested several interesting leads. The first clue was provided in 1973 by the generation of retinoblastoma-like tumors in rats following a single intraocular injection of human adenovirus (7). Although this outcome was subsequently replicated in baboons (8), the implications of these findings had to wait until the late 1980s when the binding of adenovirus proteins to cellular RB protein and p53 protein was shown to be essential for the viral transformation of animal cells (9,10). A similar result was obtained in 1990 by investigators studying the effect of the ectopic expression in mice of a simian virus 40 large T antigen (Tag) transgene. Unexpectedly, the transgene was expressed in retinal tissues, giving rise to the development of multifocal retinal tumors (11). A subsequent Tag transgene driven by a photoreceptor-specific promoter confirmed these findings, with the development of mouse retinal tumors that were indistinguishable from human retinoblastoma tumors (12). Since Tag binds (and presumably inactivates) both the RB and p53 proteins, a more elegant study examined the effect of targeting RB protein alone using the RB-specific viral oncoprotein E7 (13). In these mouse models, the targeted inactivation of RB protein alone in retinal cells resulted in retinal cell apoptosis, while the inactivation of RB using the same viral E7 transgene in a p53 (+/-) mouse resulted in multifocal retinal tumors (14,15). In summary, these findings consistently show that mouse retinal cells require the simultaneous loss of both RB and p53 activity for malignant clonal expansion. Therein, however, lies at least one dilemma when considering these animal studies as models for human retinoblastoma. Inactivation of the

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p53 pathway in human retinoblastoma by either somatic mutations or overexpression of the MDM2-binding protein has not been consistently demonstrated (16). Schlamp et al. (4), in the present study, analyzed six immortalized human retinoblastoma tumor cell lines and found abundant expression of p53 messenger RNA with a wild-type nucleotide sequence. Immunohistochemical staining, however, unexpectedly showed predominantly cytoplasmic localization of the p53 protein in four of the six tumor cell lines and cytoplasmic/nuclear co-localization in the remaining two cell lines. The conclusion of the authors is that these findings suggest p53 dysfunction through nuclear exclusion of the normally wild-type protein, as a late event in the progression of human retinoblastoma. Implicit in this hypothesis is that clinically invasive retinoblastoma does not arise from the inactivation of a single gene (RB), but instead requires the loss of at least two different gene functions. Support for the notion that RB inactivation alone is insufficient for the development of retinoblastoma tumors was suggested previously by clinical and epidemiologic observations from obligate carriers of germline mutant RB alleles who present with nonproliferating retinal scars called either retinomas (17) or retinocytomas (18). To explain these findings, it was hypothesized that the benign retinal lesions arose through the loss of RB alone, while invasive retinoblastomas would require additional (undefined) genetic hits (16,17). In addition, it is of interest that human neuroblastoma, a human tumor type that resembles retinoblastoma through greater than 90% inactivation of the RB gene (19), we have been unable to identify an immortalized RB(−/−) tumor cell line that retains wild-type p53. Therefore, the inactivation of p53 function in human retinoblastoma is biologically plausible. However, have Schlamp et al. provided convincing evidence to support this conclusion? Unfortunately, they have not directly tested whether the p53-response pathway is compromised in these cells. For example, ‘aberrant’ cytoplasmic sublocalization of p53 has been reported in breast cancer and neuroblastoma (20,21). When neuroblastoma cells expressing predominantly cytoplasmic p53 were subjected to DNA damage by γ-irradiation, an increase in nuclear p53 protein was observed that was followed by wild-type transcriptional induction of the p21 and MDM2 genes (22). Although this observation was interpreted as evidence that cytoplasmic sequestration is not a mechanism for inactivating p53 function (22), it remains possible that subjecting cells with cytoplasmically localized p53 to a more subtle stress might unmask important qualitative or quantitative (including lag time) differences in the p53 response. The challenge, therefore, is to address these issues directly and to define the molecular basis for the basal cytoplasmic localization of p53 as a late event in these selected tumor samples. While evidence that loss of p53 function is required for the pathogenesis of human retinoblastoma would narrow the gap between human and mouse models for retinal tumors, it would not necessarily answer the initial question of why humans are the only known species that develop retinoblastoma spontaneously. Is there something unique in the development of the human embryonic retina that requires or facilitates post-translational inactivation of the p53 pathway in a subset of specialized cells during a narrow window of vulnerability? A definitive answer will help in understanding the molecular basis of this rare pediatric tumor and in defining additional important connections between the RB and p53 tumor suppressor pathways.

References