The Retinoblastoma-Like Protein Family: Still in the Shadow of the RB Gene?

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Exactly 10 years ago, the field of cancer biology received a quantum boost with the realization that viral proteins from three unrelated DNA tumor viruses had evolved the capacity to transform cells by binding to a set of cellular proteins that included the retinoblastoma (RB) tumor suppressor gene product (1–3). From a biochemical perspective, this observation allowed the development of a working model where RB can mediate growth inhibition or promote cellular differentiation by reversibly binding to an increasingly complex list of cellular proteins (4,5). From a clinical perspective, this observation has led to the identification of several promising targets for cancer treatment (6), but it has also raised the controversial question of what role ubiquitous DNA tumor viruses may play in human disease (7–9). Over the past several years, two other RB-like gene products, designated RBL1/p107 (called RBL1 here) and RBL2/pRB2/p130 (called RBL2 here), have been isolated (10–13). Although the RBL1 and RBL2 genes are structurally related and have each conserved the ability to bind to a similar group of nuclear transcription factors, our understanding of how they interrelate with each other is still undefined (5,14,15). For example, while each member of the RB-like family can suppress cell growth in vitro (16–19), the RB gene has convincingly met criteria for a tumor suppressor gene (20).

A study by Howard et al. (21) reported in this issue of the Journal directly addresses the question of whether the forced overexpression of the RBL2 gene can suppress in vivo growth of a virally transformed tumor cell line with the use of a novel animal model system. Their findings serve to highlight the unanswered questions of how the RBL1 and RBL2 genes relate with the RB tumor suppressor pathway and whether they are, in fact, bona fide tumor suppressor genes. Howard et al. (21) transfected the RBL2 gene under the control of an efficient tetracycline-responsive promoter into a JC virus-transformed hamster brain tumor cell line. Since the JC virus-transforming viral T antigen (TAg) binds and presumably inactivates all RB-like gene products, including RBL2, Howard et al. initially selected a parental JC virus-transformed cell line clone that expressed TAg to prevent tumorigenesis. While the sustained expression of TAg proteins of DNA tumor viruses may participate in the development of a range of human cancers by binding to key cellular proteins, including RBL2. While it is beyond the scope of this editorial to comprehensively deal with the two issues previously mentioned, several recent observations that interconnect these hypotheses are worth noting.

The reason why both the RBL1 and RBL2 genes have stayed in the shadow of the RB tumor suppressor gene is straightforward. While RB−/− knockout mice are embryonic-lethal and mutational inactivation of RB can be identified readily in a subset of human cancers, RBL1 and RBL2 murine knockouts are viable, and there are, to date, few data demonstrating that these related genes are targeted for mutations in human cancers (14,22). To avoid semantic arguments over the definition of a tumor suppressor gene, a relatively inclusive definition was proposed recently that required the unequivocal presence of “loss-of-function” mutations in tumor samples (20). It is noteworthy, therefore, that, instead of an RBL2−/− tumor cell line, Howard et al. (21) tested a JC virus-transformed tumor cell line for RBL2 suppression. In this model, the authors imply that the high levels of RBL2 induced with their plasmid expression system could overcome the TAg-mediated transformation by the JC virus.

Although this is a reasonable scenario, it does not exclude the possibility that RBL2 induction may suppress tumor growth by overwhelming the ability of TAg to interact with non-RBL2 targets, such as RB. The statement by Howard et al. (21) that they have identified human tumors with RBL2 mutations (cited as “unpublished data”), therefore, should now allow the direct testing of the effect of RBL2 replacement and will provide the missing confirmation that RBL2 is a “classic” tumor suppressor gene.

In addition, defining the role of the RBL2 gene in human cancer has implications that extend beyond the RBL2 gene itself. For example, studies of simian virus 40 (SV40) transformation show that an N-terminal domain of SV40 TAg, referred to as the “J domain,” is essential for selectively inactivating the functional activity of RBL1 and RBL2 and for inducing a fully transformed phenotype, even in RB−/− cell lines (23,24). These findings suggest that, under certain conditions, loss of RB function alone is not sufficient for the transformed phenotype, and a complex cooperation may exist between RBL1, RBL2, and/or RB to prevent tumorigenesis. While the sustained expression of viral TAg may simultaneously inactivate all three RB-like members, it is also likely that one of the most common acquired mutations in human cancer, inactivation of the cyclin-dependent

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kinase-4 inhibitor (CDKN2a), also serves to accomplish this goal. Therefore, it is surprising that human mesothelioma, which has been linked most tightly with SV40 infection (7), also demonstrates 100% inactivation of the CDKN2a gene in primary tumors (25), which raises questions about the role of TAg in these tumors.

In summary, the requirement for RBL1 and RBL2 inactivation to unmask the fully transformed phenotype of virally infected animal cells (23,24) and other recent developments in double-gene knockout murine models (14) are exciting findings that are beginning to lead these RB-like members out of the shadow of the RB gene. Focusing higher scrutiny on these genes is overdue, especially if one considers that a phylogenetic analysis of RB-like genes from the GenBank/European Molecular Biology Laboratory (EMBL) databases were subjected to a clustalw alignment and trees were constructed by use of two different algorithms: either the pauptree (A) or pileup (B) programs (GC/GX/Oxford Molecular Company, Madison, WI). Human (document identification No. 190958, hRB), murine (132165, mRB), chicken (631029, chRB), Xenopus (348583, xcrB), and newt (1666661, nrB) homologues for the RB gene; human (1172848, hRBL1) and murine (1871224, mRBL1) homologues for RBL1/p107; human (138150, hRBL2), murine (1255232, mRBL2), and rat (2760811, rRBL2) homologues for RBL2/p130; Drosophila RB-like gene (1403031, RBF); Caenorhabditis elegans RB-like gene (1946998, ceRB); maize RB-like gene (2352795, zm1RB).

REFERENCES


