Who Is the Boss in the Retinoblastoma Family? The Point of View of Rb2/p130, the Little Brother

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Abstract

This review portrays an updated overview about the possible tumor suppressive properties of the Rb2/p130 gene, the third member of the retinoblastoma (RB) family of genes, including RB itself and p107. After a brief analysis of the established structural and functional similarities among the three genes, the main purpose is to critically analyze present evidence whether Rb2/p130 shares the role of a tumor suppressor. Taking into account the well-proven growth suppressive properties of Rb2/p130 and p107, we discuss the analysis of mutated or deleted forms of Rb2/p130 found in a number of human cancers. Finally, we take into consideration the data provided by the targeted disruption of each RB family gene, alone or in combination, in the mouse model.

Introduction

The identification of the tumor suppressor genes, also known as oncosuppressor genes or antioncogenes, represents a crucial milestone in the understanding of cancer genetics (1). The archetypal tumor suppressor is frequently represented by the retinoblastoma (RB) gene, the loss-of-function of which is responsible for the susceptibility to retinoblastoma, a sporadic or hereditary pediatric neoplasm arising from retinal cells harboring either deletion or mutational inactivation of both RB alleles (2–4). Indeed, RB complies with all of the requirements to be biologically defined as a bona fide tumor suppressor gene. In fact, its mutations or deletions are shared by several malignancies, and, in addition to this sine qua non, exogenous expression of wild-type RB in RB-defective cancer cells promptly reverts main characteristics of the neoplastic phenotype (Refs. 5, 6 and references therein).

The RB gene is now considered the founder of the RB family, because two other genes that are structurally and functionally related, namely p107 (7, 8) and Rb2/p130 (9–11) have been identified more recently. A common relevant biological activity shared by the three members of this family is the ability to negatively control the cell cycle (8, 12–14). In fact, they negatively modulate the transition between the G1 and S phases, using mechanisms mostly related to inactivation of transcription factors, such as those of the E2F family, that promote the cell entrance into the S phase (5, 6, 15, 16). Evidence has been gathered during the last decade that, in addition to the cell cycle, the RB family regulates a wide spectrum of complex biological phenomena, such as differentiation, embryonic development, and apoptosis (Refs. 17–20 and references therein).

In light of the significant structural and functional similarities among the three members of the RB family, one can argue that p107 or Rb2/p130, known to be potent cell cycle inhibitors, could also act as tumor suppressors. However, cell cycle arrest is necessary but not sufficient evidence to consider each RB family member as an oncosuppressor. In fact, without further biological information, this hypothesis could turn out to be nothing more than an easy generalization.

Cytogenetically, Rb2/p130 maps to the region 16q12.2–13, an area repeatedly altered in human cancers (10, 11, 21), whereas p107 maps to the human chromosome region 20q11.2, a locus not frequently involved in human neoplasms (7). To date and to the best of our knowledge, there is only a recent report (22) of deletion or functional inactivation for p107 in human tumors. An extensive study to search for additional mutations in the p107 sequence would be definitely advantageous in this context. It should be remarked, however, that p107 suppresses the development of RB in RB-deficient mice (23). Conversely, Rb2/p130 is shown to have tumor-suppressor properties in JC virus-induced hamster brain tumor cells (24), and mutations of the Rb2/p130 gene have been often detected in human cancers (Table 1).

Under these premises, we feel that, to date, the question of whether the RB family of genes harbors other bona fide tumor suppressors could be reasonably addressed only for Rb2/p130. This does not exclude the possibility to draw a similar hypothesis for p107 in the future.

We will briefly itemize arguments in favor of and against the possible role of Rb2/p130 as a tumor suppressor in the attempt to draw some constructive comments.

Pro

Rb2/p130 Down-Modulates the Cell Cycle. Undoubtedly, Rb2/p130 gene and its product, pRb2/p130, are structurally and functionally similar to the RB gene and protein, respectively (6). In addition, Rb2/p130 has been shown to block the cell cycle in vitro (14), whereas RB overexpression sometimes fails to do so (25). These features of Rb2/p130 are shared with p107.

Rb2/p130 Gene Product Expression Is Inversely Correlated with Cancer Malignancy. Immunohistochemical analysis of pRb2/p130 in a wide spectrum of human malignancies shows that progressive down-regulation of pRb2/p130 correlates with tumor grading and with negative patient outcome in lung cancer (26, 27), in endometrial carcinoma (28), in oral squamous cell carcinoma (29), and in uveal melanoma (30). In these systems, pRb2/p130 is a good candidate to be a molecular marker for tumor grading, because its expression is inversely correlated with tumor progression. These findings are strongly in accordance with the growth suppressive properties of pRb2/p130 at least. In fact, it is conceptually correct to assume that pRb2/p130 down-regulation allows cancer to replicate faster, making neoplastic cells more susceptible to accumulate additional mutations, thus leading to tumor progression.

Rb2/p130 Is Found Mutated in Cancer Cells. Rb2/p130 has been found deleted or functionally inactive in several cancer cell lines and biopsies. Starting from the “historical” report (14) of a human nasopharyngeal carcinoma cell line in which Rb2/p130 mRNA is expressed at very low levels, a number of mutations in the Rb2/p130 nucleotide

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3 The abbreviations used are: RB, retinoblastoma; MEF, mouse embryonic fibroblast; NLS, nuclear localization signal.

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sequence have been detected in other primary nasopharyngeal carcinomas, resulting in modifications of the primary protein structure that strongly impair its function (31). Recently, point mutations of Rb2/p130 have been described, leading to amino acid substitutions in the COOH-terminal domain of the protein, namely in the bipartite NLS. Such mutations produce a full-length pRb2/p130 molecule, which remains typically normal, and last but not least, not susceptible to tumor development (41, 42). Nevertheless, it has been shown recently that genetic background plays a key role in determining the outcome of p107 or Rb2/p130 deficiency. Although the mutational analyses described above have been done in a 129/Sv or 129Sv/C57BL6 background, different and more severe consequences have been observed when the same mutations are analyzed in Balb/cj mice (44). Moreover, it should be considered that the disease pattern seen in humans has been poorly reproduced in mice by these kinds of experiments (45). The different physiology, genetic predisposition, and life expectancy between mouse and humans may partly account for these differences, and these arguments support the thesis that Rb2/p130 mutations appear normal. Interestingly, p107−/−;Rb2/p130−/− double knockout mice show pronounced defects, mainly in limb development (41, 42), whereas double mutant Rb−/−;p107−/− or RB−/−;Rb2/p130−/− embryos have a phenotype resembling the RB−/− and, in addition, a shorter life span (43).

**Contra Rb2/p130 Inactivation Does Not Frankly Predispose to Cancer.** Rb2/p130-deficient mice, as mentioned above, are viable, fertile, phenotypically normal, and last but not least, not susceptible to tumor development. As a corollary, because Rb2/p130−/− phenotype does not show particular predisposition to develop cancer, Rb2/p130 could not be considered as biologically relevant as RB in preventing tumor development.

**Conclusions**

The facts mentioned above show that the RB family members, despite their common structural and functional similarities, also exert nonoverlapping functions and that p107 and Rb2/p130 could not be considered straightforwardly as tumor suppressors.

Data on the behavior of Rb2/p130 in tumor progression and the loss-of-function mutations described recently strongly support the thesis of Rb2/p130 as a tumor suppressor. Yet, the principal arguments against the tumor suppressor role of Rb2/p130 arise from the data provided by the knockout mice (41, 42). Nevertheless, it has been shown recently that genetic background plays a key role in determining the outcome of p107 or Rb2/p130 deficiency. Although the mutational analyses described above have been done in a 129/Sv or 129Sv/C57BL6 background, different and more severe consequences have been observed when the same mutations are analyzed in Balb/cj mice (44). Moreover, it should be considered that the disease pattern seen in humans has been poorly reproduced in mice by these kinds of experiments (45). The different physiology, genetic predisposition, and life expectancy between mouse and humans may partly account for these differences, and these arguments represent the main reason for the experimental application of technologies able to provide tissue-specific gene inactivation (46). Such models are considered to resemble more closely the naturally occurring events that promote the onset of the neoplastic phenotype. Additionally,

**Table 1 Cancer types in which loss of heterozygosity of the Rb2/p130 locus, downregulation, or mutations of Rb2/p130 or p107 genes have been found**

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Kind of alteration</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rb2/p130 LOH</td>
<td>LOH of region 16q12.2</td>
<td>21</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>LOH of region 16q12.2</td>
<td>21</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>LOH of region 16q12.2</td>
<td>21</td>
</tr>
<tr>
<td>Prostatic carcinoma</td>
<td>LOH of region 16q12.2</td>
<td>21</td>
</tr>
<tr>
<td>Small-cell lung cancer</td>
<td>Low to undetectable expression of gene product</td>
<td>27</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>Low expression of gene product</td>
<td>28</td>
</tr>
<tr>
<td>Choroidal melanoma</td>
<td>Low expression of gene product</td>
<td>30</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>Low expression of gene product</td>
<td>57</td>
</tr>
<tr>
<td>Vulvar cancer</td>
<td>Low expression of gene product</td>
<td>58</td>
</tr>
<tr>
<td>Small-cell lung cancer</td>
<td>Point mutation</td>
<td>36</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>SV40-mediated functional inactivation in 55% of cases</td>
<td>59</td>
</tr>
<tr>
<td>Burkitt lymphomas</td>
<td>EBV+</td>
<td>33, 57</td>
</tr>
<tr>
<td>Non-small cell lung cancer</td>
<td>Point mutation</td>
<td>37</td>
</tr>
<tr>
<td>Nasopharyngeal carcinoma</td>
<td>Point mutation</td>
<td>31</td>
</tr>
<tr>
<td>p107 mutation</td>
<td>Intragenic deletion</td>
<td>22</td>
</tr>
</tbody>
</table>

* LOH, loss of heterozygosity.

Fig. 1. Analysis of structural homologies among the RB family proteins. pRb, p107, and pRb2/p130 are aligned according to amino acid sequence homology. Blue boxes, highly homologous regions among the three proteins; these boxes correspond essentially to the “A” and “B” pocket domains. Red boxes, homologous regions shared only between p107 and pRb2/p130. White boxes, nonhomologous regions. Graphic representation obtained using the MACAW 2.0.5 software (National Center for Biotechnology Information, NCM, NIH, Bethesda, MD).
because it is reasonable that mutations early in development could activate alternative pathways, there might be a substantial difference between gene mutations or deletions transmitted through the germ line and those that are somatically acquired during the life of an individual.

Another key topic is that the normal phenotype associated with the targeted disruption of either p107 or Rb2/p130 is mainly attributed to the ability of these genes to functionally overlap (47). The loss of one of these two genes may result in functional compensation by the other. The hypothesis of mutual compensation, which is also reinforced by the results of evident damages in p107−/−;Rb2/p130−/− double knockout mice (42), appears to be more conceivable, rather than a supposed dispensability of the two genes in embryonic development or in cancer surveillance.

Analysis of structural similarities among the RB family members discloses that p107 and Rb2/p130 are more strictly related between each other rather than to RB itself (Fig. 1; Refs. 6, 16). Another feature of p107 and Rb2/p130 is that their gene products display distinct timing in the interaction with the E2F family of transcription factors during either cell cycle (16, 48, 49) or differentiation (50, 51). This leads to the hypothesis that p107 and Rb2/p130 have important and similar roles, even if exerted at different functional states of the cell, and that these proteins are so essential for the cellular machinery that the presence of at least one of them is required during most of the cellular physiological functions (47).

In other words, this could be epitomized by the possibility that p107 and Rb2/p130 are similar genes with different transcriptional regulation, in order that their essential function can be modulated independently throughout complex cellular processes, such as cell cycle, differentiation, development, or apoptosis. In addition, because of the high structural similarity between p107 and Rb2/p130 gene products (Fig. 1), one can predict that additional mutations of the p107 gene leading to its functional activation could be detected shortly.

In a recent work (52), a screening of the RB family proteins, performing Western blot analysis for pRb2/p130 in 69 human lung cancer cell lines and also nucleotide sequencing of exons 19–22 of the Rb2/p130 gene in 11 human lung cancer cell lines, was carried out. The authors conclude that mutational inactivation of pRb2/p130 or p107 is a rare event in lung cancer. Nevertheless, it should be considered that Western blot analysis is the most abundant source of information for the status of the Rb2/p130 gene product in this screening. Yet, these data are only in part comparable with immunohistochemical results showing in tumors either pRb2/p130 down-regulation or extranuclear localization of a mutated protein that displays the same molecular mass of the wild-type protein.

Lately, a nonoverlapping role in p16INK4A-mediated cell cycle arrest has been pointed out by comparing the properties of primary MEFs lacking either one of each RB family members or both p107 and Rb2/p130 (53). Indeed, these findings demonstrate that pRb needs a second, nonredundant function of p107 or pRb2/p130 to accomplish full p16INK4A-mediated cell cycle arrest in G1. Moreover, very recently (54, 55), targeted disruption of the three RB genes, up to the generation and characterization of triple knockout MEFs, was carried out. Results from these works provide additional precious data on the degree of functional overlap among the three RB family members, highlighting the specific importance of p107 and Rb2/p130 in cell cycle regulation and in cellular immortalization and transformation. Targeted disruption of the three RB genes produces a phenotype that greatly differs from single or double knockout control cells. In fact, triple knockout MEFs do not arrest in G1 after contact inhibition, serum starvation, or DNA damage. Moreover, these cells do not undergo differentiation or senescence and are highly prone to transformation by the Ras oncogene. These data emphasize once again the pivotal role of RB but highlight the importance of p107 and Rb2/p130 as a minimum in cells with altered RB function, thus further specifying their active contribution to the control of tumor progression. At this point, an ultimate response for the oncosuppressor role of Rb2/p130 is still not easy to achieve. When compared with RB, the main difference is that Rb2/p130 shows a major responsibility in tumor progression, whereas its role in tumor initiation remains unanswered (Fig. 2).

Under an efficient surveillance system, mutations or deletions of growth-controlling genes would simply result in cell death, but in a few particular cases these events would lead to the initiation of neoplasia. After the initial transforming event, the accumulation of mutations in oncogenes and of mutations/deletions in tumor suppressor genes results in rapid progression to malignancy.

Initiation and progression are of course among the cardinal features of malignancy. Many studies have stressed the importance of agents that initiate tumorigenesis, but others, such as those on hereditary nonpolyposis colorectal carcinoma or on the p53 gene (56), suggest that agents that facilitate or accelerate progression are important as well.

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References


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