We Contain Multitudes: The Protean Face of Retinoblastoma

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The precise cellular characteristics of retinoblastoma have long been debated. In this issue of *Cancer Cell*, McEvoy et al. reveal that retinoblastomas are highly homogeneous at the molecular level and coexpress genes characteristic of retinal progenitors and various different mature retinal cell types, while ultrastructurally resembling amacrine cells.

Both the cellular characteristics and the cell of origin of retinoblastoma have been hotly disputed (Nichols et al., 2009). Primary retinoblastomas not only express genes characteristic of retinal progenitor cells, but also multiple different terminally differentiated retina cell types, including rod and cone photoreceptors, retinal interneurons, and glia. The gene expression profiles of all major retinal cell types have been characterized (Blackshaw et al., 2004; Trimarchi et al., 2008), and it should be possible to clarify the molecular characteristics and perhaps even the cell of origin of retinoblastoma using similar approaches. While the early postnatal presentation of retinoblastoma suggests that it may arise as a result of mutations in mitotic retinal progenitor cells, a pair of recent studies has suggested retinoblastomas can develop from postmitotic horizontal interneurons and cone photoreceptors (Ajioka et al., 2007; Xu et al., 2009). Profiling gene expression in multiple samples may resolve this confusion. Furthermore, a clear and comprehensive picture of the gene expression profile of retinoblastomas could help considerably in the design of supplemental treatments for patients who do not respond to conventional surgical and chemotherapy-based treatments.

In this issue of *Cancer Cell*, McEvoy et al. (2011) have undertaken this task with aplomb and come up with some surprising findings. By conducting microarray analysis of 52 primary retinoblastoma tumors obtained from patients and 120 tumors obtained from six different lines of mutant mice that spontaneously develop retinoblastoma, they found that the gene expression patterns of tumors from both humans and mice were very similar. The vast majority of tumors coexpressed markers of retinal progenitors, rod and cone photoreceptors, and both amacrine and horizontal interneurons, echoing results from a smaller previous survey of retinoblastoma gene expression (Ganguly and Shields, 2010). They next assessed the gene expression profile of 20 different isolated single cells obtained from xenografts of a human primary retinoblastoma. Remarkably, the gene expression profiles of individual cells closely mirrored those of the whole tumor, confirming that virtually every published study on the molecular characteristics of retinoblastoma is at least partially correct. Remarkably, however, retinoblastomas turned out to be highly homogenous at the cellular level, and uniformly coexpressed genes that are normally completely specific to various different retinal cell subtypes.

Which retinal cell type do these "hybrid" cells most closely resemble? To address this question, McEvoy et al. (2011) harnessed the power of electron microscopy to extract ten diagnostic morphological parameters for the major retinal cell types. Applying these criteria, they reported that retinoblastomas most closely resemble the amacrine cells, while the presence of large dense core synaptic vesicles suggests that they resemble one of the rare monoaminergic subtypes. These ultrastructural studies further confirmed the relative cellular homogeneity of the tumors, with no cells showing either the apical cilia or outer segments that are characteristic of rod and cone photoreceptors.

The authors conclude their study with a powerful demonstration of the potential clinical relevance of their encyclopedic analysis of the molecular and ultrastructural anatomy of retinoblastoma. Since retinoblastomas express genes characteristic of monoaminergic amacrine cells, they tested the effect of chlorpromazine and fluphenazine, which are broad-spectrum neuroleptic compounds that block both dopamine and serotonin receptors and are normally used in treatment of schizophrenia and bipolar disorder, and found that these effectively inhibited tumor growth (Figure 1).

This work is a technical tour de force, and sheds unexpected light on several different questions in both oncology and developmental biology. The fact that retinoblastoma cells robustly coexpress genes that are characteristic of at least four different mature retinal cell types, in addition to retinal progenitors, does not directly implicate any one of these cells as the source of the tumor (Figure 1). However, one notable exception comes from the tumors of $Rb^{+/-}$; $p107^{-/-}$; $p130^{-/-}$ mice, which have been previously reported to give rise to retinoblastoma through dedifferentiation of horizontal cells (Ajioka et al., 2007). The expression profile of these tumors much more closely resembled that of horizontal and amacrine cells than did that of other retinoblastomas. The cell of origin may thus indeed be atypical for this genotype, with the vast majority of tumors arising from another source, most likely retinal progenitor cells.

The two neuroleptic drugs shown to be effective in inhibiting tumor growth are unusually broad spectrum, antagonizing dopamine, serotonin, and adrenergic receptors. More specific receptor

antagonists had no effect on tumor growth. Previous studies have implicated serotonin in this role in a number of other tumor types, although this has not been reported for neuroectodermal tumors such as retinoblastoma. This may imply that multiple monoamines act synergistically as autocrine mitogens for retinoblastoma cells, a hypothesis supported by the authors' finding that retinoblastomas secrete a range of different monoamines. However, they did not show that monoamines directly promote tumor growth, and it is thus possible that these drugs may act on unexpected target sites. The finding that antipsychotic drugs may inhibit the growth of a broad range of tumors by inhibiting cholesterol metabolism (Wiklund et al., 2010) may prove informative in this respect. While this does not diminish the potential clinical usefulness of these compounds in treating retinoblastoma, much more work needs to be done to clarify their mechanism of action.

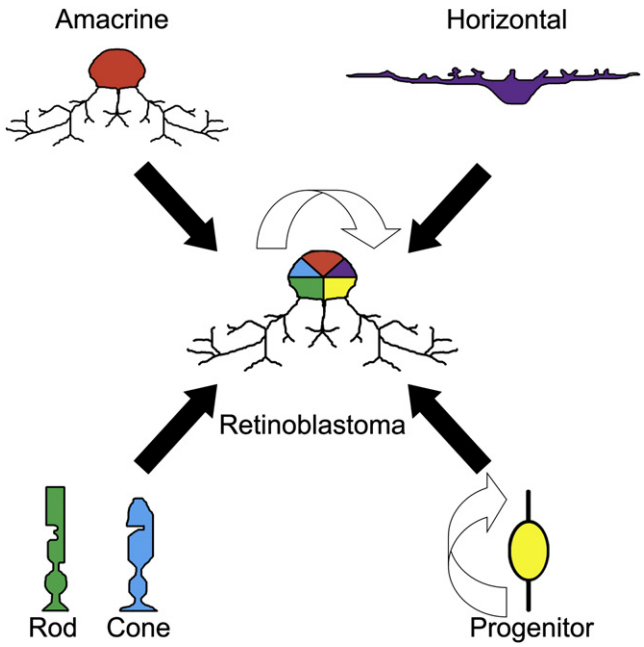

Figure 1. A New View of Retinoblastoma
Retinoblastoma cells morphologically resemble amacrine cells but simultaneously coexpress genes specific to horizontal and amacrine interneurons, rod and cone photoreceptor, and retinal progenitors.

The finding that retinoblastoma cells robustly coexpress gene characteristic has potentially far-reaching implications for developmental neurobiology. The control of cell fate specification is ultimately a process of establishing mutually exclusive, cell-specific patterns of transcription. Hundreds of different genes have been identified that regulate the process of cell fate specification, but these generally act to direct a cell to adopt one fate in preference to another, or to drive differentiation from a less to a more differentiated state. Loss of function of a handful of transcription factors and coregulators has been previously found to result in hybrid retinal cell types, but these findings come with some important qualifications. Such mutant cells coexpress genes from only two cell types, such as rod and cone photoreceptors, which are closely functionally related in any case, with only a subset of cell-specific genes misregulated (Swaroop et al., 2010). In contrast, retinoblastoma cells appear to express a full complement of genes characteristic of at least four terminally differentiated retinal cell types, as well as mitotic retinal progenitors. This data points to a central and general role of Rb family proteins in repressing inappropriate expression of cell type-specific genes.

What exactly is going on, and what relevance might it have to the question of the retinoblastoma cell of origin? The fact that retinoblastomas do not express markers of the bipolar interneurons and Muller glia—two last-born retinal cell types—offers one potential clue. This result is consistent with a model where loss of function of Rb family proteins in early-stage retinal progenitors might both prevent cell cycle exit and also compromise repression of terminal markers of early-born cell types. Clues to how this might happen are provided by the finding that Rb acts in mesenchymal stem cells to regulate cell fate specification by directly binding and modulating the activity of developmentally important transcription factors (Calo et al., 2010). Further analysis of the targets of Rb family proteins will shed light on whether and how they prevent one retinal cell type from simultaneously becoming many.

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