expand the potential clinical applications of this vector. Most importantly, this work convincingly demonstrates that ectopic expression of a single miRNA can markedly impact a disease, in this case hepatic cancer. AAV vectors can be produced in large quantities making it highly suitable as a gene therapy vector. Given that AAV vectors do not integrate into the host genome, they will eventually be eliminated, thereby minimizing the potential of vector-mediated toxicities. What is truly exciting about this study is the strong correlation between miR-26a expression and suppression of hepatic cancer. Restoring miR-26a levels in hepatic cancer cells via transduction with AAV vectors is clearly a path to the clinic that will be exploited given the high prevalence of liver cancer worldwide. The use of a natural miRNA to suppress cell proliferation is exciting and hopefully will lead to a new therapeutic strategy for the treatment of liver cancer.

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Retinoblastoma, an Inside Job

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Why are some cell types more prone to transformation than others? In this issue, Xu et al. (2009) show that retinoblastoma cells co-opt several intrinsic features of cone photoreceptors for their survival and growth.

Successful thieves select targets carefully, knowing that a well-guarded establishment is unlikely to surrender the loot. Oncogenic insults are like thieves in this regard, as their effect is highly dependent on cell type. Their mission is more likely to succeed in a cell where the defenses, such as death, terminal differentiation, senescence, or sensitivity to immune surveillance, are weaker. The last few decades have exposed most of the culprits, a scurrilous cast of activated oncogenes and inactivated tumor suppressors, but the cell-specific weaknesses—the inside crew—that allow them to flourish are more mysterious.

Now, David Cobrinik and colleagues redress this balance for the childhood ocular cancer, retinoblastoma (Xu et al., 2009). This tumor is initiated by inactivation of both copies of *retinoblastoma 1* (*RB1*) and helped along by subsequent genetic events (Figure 1A) (Corson and Gallie, 2007). Xu et al. now expose a quartet of insiders that help these oncogenic events to take hold: N-Myc, MDM2, and the nuclear receptors RXRγ and THRβ2 (Figure 1B). Although their presence is essential for tumorigenesis, they are not altered by any genetic, epigenetic, or posttranscriptional events typically associated with oncogene activation.

To expose retinoblastoma's collaborators, the authors first defined the predominant cell type in retinoblastoma. Prior studies generated conflicting conclusions, failing to distinguish normal versus tumor cells. This issue is elegantly resolved by Xu et al., who in one approach follow RB protein to mark normal cells, and in an even better strategy select tumors with a homozygous *RB1* deletion and used fluorescent in situ hybridization to distinguish null tumor from normal cells. Colabeling reveals that most *RB1*-deficient tumor cells resemble cone photoreceptors, the cells that allow us to see color. Markers present include the retinoic acid-activated nuclear receptor RXRγ and the homeodomain protein CRX (which are also expressed in some other retinal neurons) as well as the cone-specific thyroid hormone-activated nuclear receptor, THRβ2, and two opsins. Humans have three types of cones with distinct opsins that detect blue (short wave, S), green (medium wave, M), and red (long wave, L) light. Xu et al. show that retinoblastoma is replete with markers of M/L cones. This fits well with the presence of THRβ2, which blocks S and promotes L/M cones (Ng et al., 2001).

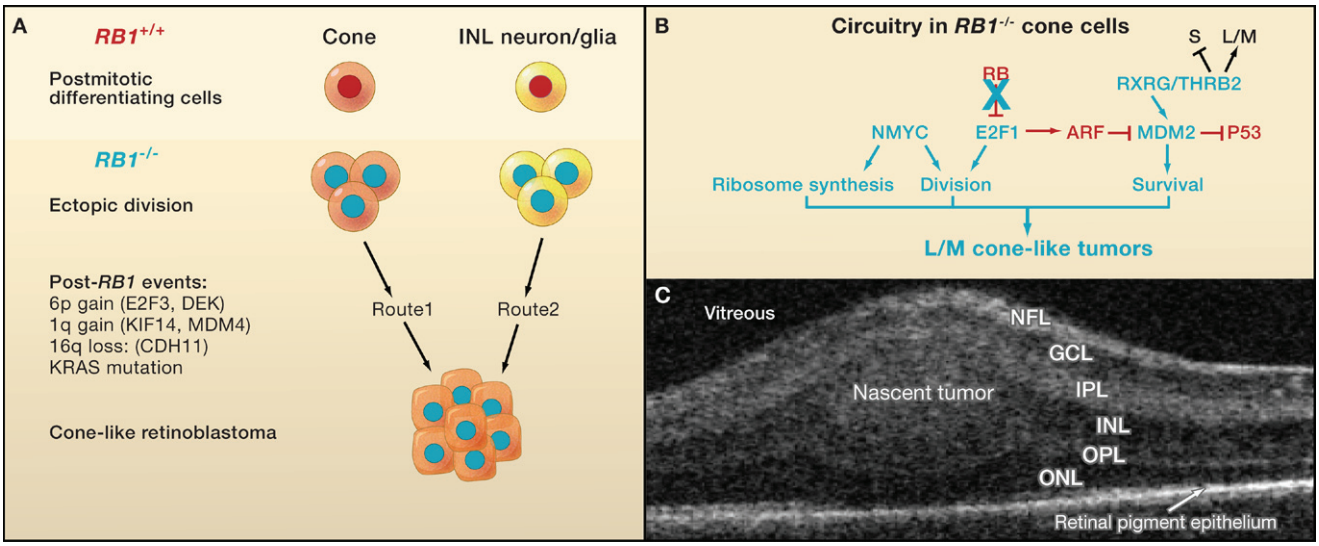

Figure 1. Retinoblastoma's Accomplices

(A) *RB1* loss initiates ectopic division (blue nuclei). Tumor progression is driven by the subsequent changes indicated. Normal aspects of cone circuitry cooperate with these alterations. Tumors might exploit these features of cone precursors (Route 1), but may reroute to a cone fate after initially exploiting features of another cell type (Route 2).

(B) E2F1 sparks ectopic division of differentiating cells and can also activate p53 through various pathways, including induction of *ARF*. Intrinsically high levels of MDM2 counter that effect, promoting survival. High levels of N-Myc, perhaps important in normal cones to drive ribosome synthesis, are co-opted along with E2F to drive division in retinoblastoma. The nuclear receptors RXRγ and THRβ2 (which may interact) are involved in cone development. THRβ2 inhibits synthesis of S cones, which respond to short wavelength light, promoting differentiation of cones that respond to medium and long wavelengths. RXRγ and THRβ2 are also required for survival and possibly division and other tumorigenic functions in retinoblastoma. Red, antitumorigenic factors; blue, protumorigenic factors/events; black, neutral factors.

(C) Imaging of the retina of an *RB1*$^{+/-}$ child a few weeks after birth by live Bioptigen fourier domain optical coherence tomography (kindly provided by A. Mallipatna, C. Vandenhoven, B.L. Gallie, and E. Héon). The retinal pigment epithelium lines the back of the eye. Photoreceptors (cones and rods) in the outer nuclear layer (ONL) form synapses in the outer plexiform layer (OPL) with interneurons in the inner nuclear layer (INL). These cells then form synapses in the inner plexiform layer (IPL) with neurons in the ganglion cell layer (GCL). Finally, ganglion cells generate a nerve fiber layer (NFL) that connects to the brain. The nascent tumor is emerging in the INL, rather than the ONL.

In mice, *Rb1* loss causes abnormal division of differentiating retinal cells (Chen et al., 2004; MacPherson et al., 2004). If we assume that the fetal human retina responds similarly to *RB1* deficiency, a critical question emerges: what advantages does the L/M cone circuitry offer a tumor that other retinal cells do not?

The first suspect nabbed is the infamous MDM2, which can counter E2F1-induced death. Given that the absence of RB triggers E2F1-dependent division in differentiating retinal neurons and concomitant apoptosis in a subset (Chen et al., 2007), MDM2 could be useful for an aspiring retinoblastoma cell. *MDM2* is often amplified in human tumors with wild-type p53, but copy number is unaffected in retinoblastoma. Others report amplification of the *MDM2*-relative *MDM4* in retinoblastoma (Laurie et al., 2006), but Xu et al. report two copies in most of their samples. Strikingly, in normal development, fetal human cones the levels of MDM2 are intrinsically high. This finding invokes an attractive model in which high levels of MDM2 in retinoblastoma reflects cone identity rather than oncogenic mischief.

Xu et al. reveal that cones and retinoblastoma also share high levels of N-Myc. Knockdown of MDM2 or N-Myc impairs division and increases apoptosis in retinoblastoma cell lines. Reducing ARF levels limits the effect of MDM2 knockdown, suggesting that MDM2 acts via the ARF-p53 pathway (Figure 1B), confirming the relevance of p53 inactivation to retinoblastoma (Laurie et al., 2006). MDM2 expression in retinoblastoma requires recruitment of the nuclear receptor RXRγ to the *MDM2* promoter, and strikingly, RXRγ knockdown reduces MDM2 expression and retinoblastoma cell survival.

Of the four factors revealed by Xu et al., THRβ2 is the only one whose function is probed in an orthotopic xenograft assay, which shows that tumor growth is reduced in the absence of THRβ2. Both thyroid and retinoic acid receptors are important for cone development, and the *MDM2*-inducing function of RXRγ helps explain its tumorigenic value, but how is THRβ2 involved? Thyroid hormone has a long-standing but complex association with cancer. THRα is the cellular version of the v-ErbA oncogene, but v-ErbA and altered versions of THRβ found in human cancer are dominant negatives, suggesting a tumor suppressor function for unaltered THRβ (Guigon and Cheng, 2009). Given that THRs can affect division, differentiation, and migration, and interact with RXR and various oncoproteins and tumor suppressors (such as β-catenin, a PI3K regulator, Cyclin D1, RB, and p53), THRβ2 could promote retinoblastoma in diverse ways.

The value of N-Myc/MDM2 to retinoblastoma is obvious, but why do postmitotic cones retain these proteins? The role of MDM2 in survival could be useful in these long-lived neurons. Myc proteins target thousands of genes regulating many processes, including ribosome biogenesis, and cones need millions of opsin molecules to feed their hungry outer segments. One wonders how N-Myc-

deficient rods cope, which are larger in size than cones and need 100,000,000 opsins/cell.

The unaltered insiders discussed above are altered in other cancers; thus, whether abnormal or normal activity of a particular gene contributes to tumorigenesis is context dependent. There must also be genes that are expressed in one cell, where they counter transformation, and are naturally off in a neighbor, increasing its susceptibility. Discovering the basal unaltered features of different cell types that render them more cancer prone will expand the repertoire of potential therapies. Several labs are already testing MDM2/4 therapeutics, and if thyroid hormone is required for cancer-promoting activity of THRβ2, perturbations in its transport or activation may also be helpful.

The cone phenotype of retinoblastoma suggests, but does not prove, that the disease originates from cones. Passengers disembarking from a plane wearing winter coats imply a chilly origin, but equally could signify a frigid destination. Guessing where cancer starts from its end-stage appearance is equally ambiguous. If retinoblastoma arises from cones, one would expect more tumors at the fovea, a cone-rich region located within the posterior (central) human retina. When newborn children with heterozygous loss of *RB1* are tracked, nascent tumors do appear first at the posterior pole, but not preferentially at the fovea, and tumors also arise later in the periphery (B. Gallie, personal communication), which fits the posterior-anterior developmental wave rather than a cone origin. Cones in the outer retina synapse with interneurons in the inner nuclear layer. Tumors have been observed emerging here (Figure 1C), which may arise from displaced cones, or various interneurons or Müller glia in the inner nuclear layer. Retinoblastoma in mice also arises in the inner nuclear layer, exploiting the intrinsically high resistance of amacrine or horizontal neurons to apoptosis (Ajioka et al., 2007; Chen et al., 2004; MacPherson et al., 2004). Emerging from one ground state and converting to another seems elaborate, but altering cell type is not onerous (induced pluripotent stem cells are a topical example), and "transformation" is cancer's defining feature. Until the early response of different human retinal cells to *RB1* loss and the routes adopted during progression are understood, we remain (cones or no cones) very much in the dark. Wherever it is first employed, the cone circuitry is clearly a crucial component in retinoblastoma.

Like other breakthroughs in the study of retinoblastoma, the latest milestone has significance well beyond the eye. Normal is not the same as harmless. As genome-wide sequencing projects continue onward toward the goal of uncovering all oncogenic mutations in all tumor types, the next major step will be to expose their hidden accomplices.

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Prion Topology and Toxicity

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Inactivation of mahogunin, an E3 ubiquitin ligase, causes a spongiform encephalopathy resembling prion disease. Chakrabarti and Hegde (2009) now report that prion proteins with aberrant topologies inactivate mahogunin, providing a plausible explanation for certain aspects of prion pathology.

Transmissible spongiform encephalopathies, or prion diseases, are neurodegenerative conditions caused by prions, atypical infectious agents consisting of PrP^{Sc}, a misfolded and aggregated form of the cellular prion protein (PrP^{C}). PrP^{C} is a cell-surface GPI-anchored glycoprotein that is normally produced in abundance in brain, muscle, and the immune system (Aguzzi et al., 2008). Although we have a robust model of how prions replicate, we still do not understand how and