Nature Reviews Cancer | AOP, published online 16 October 2014; doi:10.1038/nrc3849

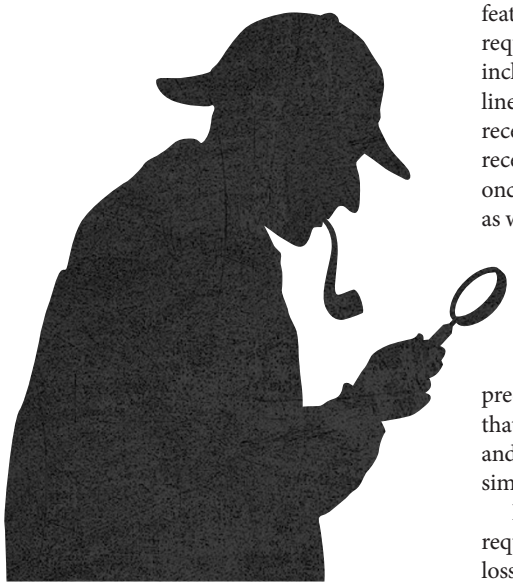
TUMORIGENESIS

Establishing the origin of retinoblastoma

> RB-depleted cone precursors and retinoblastoma cells depend on a similar signalling circuitry

Retinoblastoma is a childhood cancer of the retina that for decades has served as a model system for cancer studies. Retinoblastoma arises as a consequence of mutations in *RB1*, which encodes the retinoblastoma tumour-suppressor protein RB. Although RB is inactivated in many cancers, germline *RB1* mutations predispose to retinoblastoma far more strongly than to any other type of cancer. Now, the teams led by David Cobrinik and Suresh Jhanwar have identified the cell type that gives rise to retinoblastoma, explaining why the retina is particularly reactive to mutations in *RB1*.

To investigate the cell of origin of retinoblastoma, the authors knocked down the expression of RB in different types of human embryonic retinal cells with *RB1*-directed short hairpin RNAs (shRNAs). They then analysed the proliferation rate of these RB-depleted cells by co-staining for the proliferation-associated Ki67 marker and cell-type-specific markers. *RB1* shRNAs abrogated RB expression in all of the retinal cell types, and after several weeks, Ki67 was induced only in cells that expressed photoreceptor markers, suggesting that only precursor cells destined to become cone photoreceptors proliferate in response to *RB1* loss. This cell type-specific response was confirmed in cell populations highly enriched in cone precursors. Because cone precursors are normally differentiating and postmitotic cells, this indicated that the cell of origin of retinoblastoma is not a stem cell.



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The authors also analysed the features that retinoblastoma cells require to proliferate — which included high expression of cone lineage factors (thyroid hormone receptor β2 (TRβ2) and retinoid X receptor-γ (RXRγ)) and of certain oncoproteins (MYCN and MDM2), as well as downregulation of the cyclin-dependent kinase inhibitor p27, probably mediated by the ubiquitin ligase SKP2. All of these features were also found to be required for cone precursor proliferation, indicating that RB-depleted cone precursors and retinoblastoma cells depend on a similar signalling circuitry.

In mice, retinal tumorigenesis requires loss of *Rb1* combined with loss of retinoblastoma-like 1 (*Rbl1*) or loss of *Rbl2,* whereas in human retinoblastomas, *RB1* mutations are mainly associated with loss of *RBL2*. The authors observed that maturing human cone precursors had abundant RBL2 and minimal RBL1 expression, whereas retinoblastoma cells had barely detectable RBL2 but high expression of RBL1. Interestingly, RBL1 was required for proliferation of RB-depleted cone precursos and retinoblastoma cells, in contrast to the tumour suppressor role of RBL1 in mice. After several months in culture, RB-depleted and RB/RBL2-depleted cone precursors formed aggregates that resembled retinoblastoma cells, and RB/RBL2-depleted cultures proliferated at a higher rate and for a longer period of time than those with RB depletion alone. When transplanted into immunodeficient mice, RB-depleted and RB/RBL2-depleted cone precursors formed retinoblastoma-like tumours that resembled human retinoblastomas at the histological, structural and molecular cytogenetic levels.

These data suggest that cone precursors are the primary cell type responsible for the development of retinoblastoma and imply that cone precursor circuitry collaborates with *RB1* mutations to enable tumorigenesis. Establishing the circuitry of a cancer cell of origin may be of help in designing approaches for diagnosis and early detection.

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ORIGINAL RESEARCH PAPER Xu, X. L. *et al.* Rb suppresses human cone-precursor-derived retinoblastoma tumours. *Nature* http://dx.doi.org/10.1038/nature13813 (2014)