GETTY

GENOME INSTABILITY

RB moonlights in mitosis

Loss of the retinoblastoma (RB) tumour suppressor pathway commonly occurs in tumours and is associated with chromosome instability (CIN). The RB pathway is famous for its role in mediating G1–S cell cycle checkpoint control, but can this role alone account for its tumour suppressive function?

Loss of the RB pathway affects mitosis, so van Harn *et al.* arrested mouse embryonic fibroblasts in which the three RB family members were ablated (TKO-MEFs) in G2 by serum starvation; this induced the cyclin-dependent kinase inhibitor p21, produced DNA double-strand breaks and activated the homologous recombination repair pathway. Inhibiting DNA damage response (DDR) kinases accelerated cell cycle re-entry and progression into mitosis after release from serum starvation and downregulated p21 expression. These data indicate that serum-starved RB-deficient cells accumulate DNA damage during progression through the preceding S phase and thereby activate DDR pathways. Moreover, metaphase spreads of TKO-MEFs showed defects in centromeric sister chromatid cohesion and increased chromatid breaks, suggesting that damage persists in mitosis. Consistent with this, TKO-MEFs that re-entered the cell cycle after serum re-addition had increased copy number alterations. Sister chromatid cohesion is initiated during S phase by the cohesin complex, and the authors speculate that the RB pathway may affect proper loading of the cohesin complex.

Manning *et al.* show that downregulation of RB1 expression in TERT-immortalized RPE1 cells (which are diploid epithelial cells) using RNA interference induces aneuploidy and chromosome missegregation. RB1 knockdown delayed mitotic progression, increasing the proportion of cells in prometaphase. They also observed further mitotic defects and suggested that this might originate from a defect in the structure of the centromere, which is regulated by the cohesin and condensin complexes. Chromatin association of RAD21 — a subunit of the cohesin complex — was reduced in mitotic cells lacking RB1, suggesting that its loss impairs the loading or maintenance of the cohesin complex at the centromere.

RBF1, the *Drosophila melanogaster* RB homologue, interacts with the condensin II subunit CAP-D3. Manning *et al.* showed that RBF1 also promotes cohesin complex loading onto DNA and that CAP-D3 loading on chromatin was decreased in RB1-deficient RPE1 cells. Knock down of CAP-D3 caused similar mitotic defects in RPE1 cells to those seen on RB1 knockdown. Interestingly, condensin II complexes are enriched at the centromeres of mitotic chromosomes, suggesting that RB1 loss causes defective centromeric condensation and cohesion, which leads to chromosome segregation errors and CIN.

So, is this role of RB1 important for its function as a tumour suppressor? Coschi *et al.* used $Rb1^{\Delta L/\Delta L}$ mice that express a mutant RB1 that cannot bind condensin II complexes to test this. MEFs from these mice showed lagging chromosomes and centromeric fusions, which were not the result of loss of G1–S cell cycle checkpoint control. Live imaging revealed the same mitotic defects, which they showed caused aneuploidy from either an abrupt termination of metaphase delay (probably caused by chromosome breakage) or a failure to complete mitosis (which generated binucleated cells). Condensin II subunits were also improperly loaded onto chromatin. The expression of the RB1 mutant reduced the latency of tumour initiation in $Rb1^{\Delta L/\Delta L}$ $Trp53^{-/-}$ and $Rb1^{\Delta L/\Delta L}$ $Trp53^{+/-}$ mice. Moreover, the tumours were more aggressive, there was a higher incidence of metastases and a higher proportion of mice had multiple tumours. By comparing thymic lymphoma cells from $Rb1^{\Delta L/\Delta L}$ $Trp53^{-/-}$ and $Trp53^{-/-}$ mice the authors found that whole and segmental chromosome gains and losses were increased in the lymphoma cells that expressed mutant RB1, suggesting that the accompanying CIN could account for the reduced tumour latency.

Together, these data suggest that the CIN resulting from the inability of RB1 to maintain sister chromatid cohesion promotes tumorigenesis and that this is an important function of RB1 as a tumour suppressor.

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ORIGINAL RESEARCH PAPERS van Harn, T. *et al.* Loss of RB proteins causes genomic instability in the absence of mitogenic signalling. *Genes Dev.* **24**, 1377–1388 (2010) | Manning, A. L. *et al.* Loss of pRB causes centromere dysfunction and chromsome instability. *Genes Dev.* **24**, 1364–1376 (2010) | Coschi, C. H. *et al.* Mitotic chromosome condensation mediated by the retinoblastoma protein is tumor suppressive. *Genes Dev.* **24**, 1351–1363 (2010)