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A potential link between p53, cell competition and ribosomopathy in mammals and in *Drosophila*

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ABSTRACT

The term cell competition has been used to describe the phenomenon whereby particular cells can be eliminated during tissue growth only when more competitive cells are available to replace them. Multiple examples implicate differential activity of p53 in cell competition in mammals, but p53 has not been found to have the same role in *Drosophila*, where the phenomenon of cell competition was first recognized. Recent studies now show that *Drosophila* cells harboring mutations in Ribosomal protein (Rp) genes, which are eliminated by cell competition with wild type cells, activate a p53 target gene, Xrp1. In Diamond Blackfan Anemia, human *Rp* mutants activate p53 itself, through a nucleolar stress pathway. These results suggest a link between mammalian and *Drosophila Rp* mutants, translation, and cell competition.

P53 is a transcription factor that is well known as the guardian of the genome. When activated by DNA-damage, p53 coordinates cell cycle arrest that facilitates DNA repair, or, if damage is more extreme, apoptosis that removes irretrievably damaged cells (Levine and Oren, 2009; Vousden and Prives, 2009). In undamaged cells, baseline p53 activity is low, in part due to rapid turnover controlled by the E3 ubiquitin ligase MDM2 (Moll and Petrenko, 2003). Activity increases rapidly after modification by the DNA Damage Response kinases ATM and Chk2 (Levine et al., 2006). P53 regulates the expression of many genes, some direct targets but others indirect (Fischer, 2017).

Recently, evidence has been accumulating for another function of p53, in the process of cell competition. Cell competition involves the selective elimination of particular cells based on intrinsic differences from their cellular context, eg elimination of certain genotypes of cells from genetic mosaics that would survive and proliferate in a genetically homogenous environment (Claveria and Torres, 2016; Di Gregorio et al., 2016; Merino et al., 2016) (Fig. 1A). Cell competition does not reflect a cell-autonomous cell death process, such as can be caused high levels of p53 activity, but requires interaction with distinct neighboring cells that are fitter and able to replace the out-competed cells. Cell competition is of interest because of its possible roles in tumor development and tumor surveillance, both situations where cells of distinct genotypes confront one another and influence one another's growth and survival (Baker, 2017; Maruyama and Fujita, 2017). Cell competition may also help prevent developmental defects and monitor stem cell populations for inappropriate differentiation (Merino et al., 2016; Diaz-Diaz et al., 2017).

The classic example of cell competition is the elimination in *Drosophila* of $Rp^{+/-}$ cells from mosaic imaginal discs (undifferentiated larval tissues that contain the rapidly proliferating progenitors for adult structures) that also contain wild type cells (Fig. 1A). Another example includes competition between imaginal disc cells that express different levels of the proto-oncogene Myc. In the latter case even wild type cells can be eliminated by cells expressing more Myc ('supercompetitors'). A further example concerns cells that are mutated for the *scribbled* gene (*scrib*). The conserved Scrib protein is required for epithelial cell polarity and in *scrib* mutants the imaginal discs become neoplastic. By contrast, clones of *scrib* mutant cells are eliminated from mosaic imaginal discs by competition with the wild type cells before they become neoplastic (Claveria and Torres, 2016; Merino et al., 2016; Baker, 2017).

One of the first cell competition phenomena described from mammals involved hematopoietic stem and progenitor cells. Mild irradiation, that by itself would have negligible effect on hematopoietic stem cell viability and function, significantly disadvantages these cells for months afterwards in competitive situations where stem cells with less p53 activity also present (Bondar and Medzhitov, 2010; Marusyk et al., 2010). In a study of embryonic development, p53 knock-down ES cells injected into E3.5 day blastocysts strongly compete with co-injected control ES cells by E14.5, indicating that even the baseline p53 activity of normal blastocyst cells can be disadvantageous in the presence of cells lacking even that activity (Dejosez et al., 2013). Dejosez (2013) also identified circumstances where p53 knockdown could be disadvantageous. Mild p53 activity is also shown to be disadvantageous in studies of

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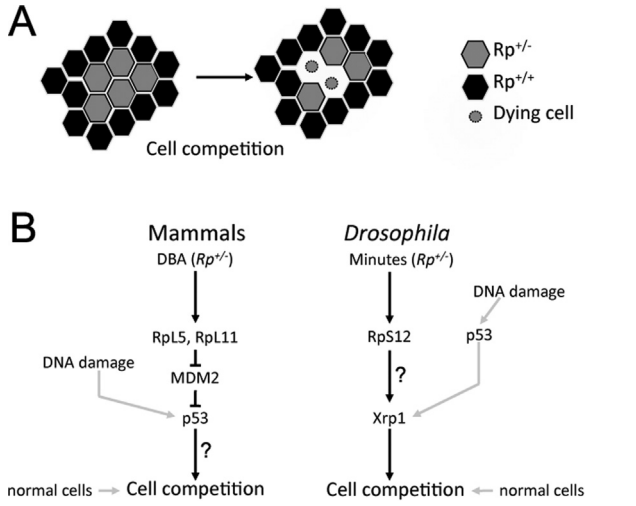

Fig. 1. A) Cell competition describes an elimination of cells that relies on a difference in their intrinsic properties compared to nearby cells. In *Drosophila*, most $Rp^{+/-}$ genotypes that are cell-autonomously associated with slow development and specific morphological defects (the so-called 'Minute" phenotype) are also eliminated from growing tissues when wild type ($Rp^{+/+}$) cells are also present. B) In mammals, activation of p53, for example by DNA damage, marks cells for elimination and replacement by cells with lower p53 activities. $Rp^{+/-}$ genotypes activate p53 through nucleolar stress that depends on RpL5 and RpL11 to inactivate MDM2, and are associated with Diamond Blackfan Anemia. It is not yet directly demonstrated that mammalian $Rp^{+/-}$ cells are eliminated by cell competition. In *Drosophila,* DNA damage activates the putative transcription factor Xrp1 through p53. $Rp^{+/-}$ genotypes activate Xrp1 through RpS12 instead, which in mosaic tissues marks these cells for elimination and replacement by wild type ($Rp^{+/+}$) cells. Both p53 in mammals and Xrp1 in *Drosophila* predispose cells to elimination by competition, if more normal cells are present.

mdm2 family members. These ubiquitin ligases are major negative regulators of p53. Whereas $Mdm2^{+/-}$ $Mdm4^{+/-}$ double heterozygous mice show only a mild increase in p53 activity and undergo normal embryogenesis, they are at a disadvantage in chimeras and outcompeted by normal cells in which p53 activity maintains a lower baseline (Zhang et al., 2017).

In tissue cultures, mammalian *Scribbled* is also involved in cell competition. *Scrib* knock-down has little effect on MDCK cells in homogenous culture, but when these cells are co-cultured with wild-type MDCK cells then the knock-down cells are selectively eliminated by apoptosis. Gene profiling led to the discovery that p53 was activated during the competition, and in fact was required for the *Scrib* knock-down cells to be eliminated. Accordingly, even in otherwise normal MDCK cells, mild p53 activity by itself was sufficient for these cells to be eliminated in mixed culture (Wagstaff et al., 2016). P53 activity is also found to be required for competitive elimination of mouse embryo cells mutated for *Bmpr1a*, and for tetraploid cells (Bowling et al., 2018).

In summary, p53 activity is a common feature of cell competition in mammals. In addition to cell-autonomous roles in cell cycle arrest and apoptosis that follow DNA damage, low levels of p53 activity that normally are compatible with cell growth and survival have an effect in chimeras or mixed cultures where other cells are present that have lower p53 activity levels. In some cases, such as competition between MDCK cells with and without *Scrib* expression, or mouse embryo cells that are tetraploid or mutated for Bmpr1a, changes in p53 activity are induced by other genetic changes.

Until recently there had been little evidence for any molecular similarity between what little was known of the mechanisms of cell competition signaling in *Drosophila* and mammals (Baker, 2017; Maruyama and Fujita, 2017). In particular, *p53* is not required in $Rp^{+/-}$ cells for their elimination from mosaic imaginal discs (Kale et al., 2015), and cells lacking *p53* do not eliminate wild type cells in *Drosophila*(de la Cova et al., 2014). The *p53* gene is also not required in wild type cells for their elimination by cells expressing more Myc. P53 does seem to have another effect. In cells expressing more myc: under competitive conditions, it shifts their metabolism and promotes their survival and ability to eliminate nearby wild type cells (de la Cova et al., 2014).

Recently, however, a hint that cell competition in mammals and *Drosophila* might share something in common has emerged. $Rp^{+/-}$ cells that undergo cell competition in *Drosophila* elevate expression of a bZip domain protein, Xrp1, which contributes to many of the altered properties of $Rp^{+/-}$ genotypes. This includes their reduced overall translation rate, slower cellular growth rate, and slower rate of organismal developmental, as well as their susceptibility to elimination by cell competition (Lee et al., 2018). Importantly, the *Xrp1* locus had already been identified as the most highly-induced transcriptional target of p53 following irradiation (Brodsky et al., 2004). Xrp1 may contribute to the DNA damage response downstream of p53, since it is required for aspects of genome stability (Akdemir et al., 2007). Multiple genes that were previously described as p53 targets are upregulated in $Rp^{+/-}$ cells in an *Xrp1*-dependent manner, suggesting that they may actually be Xrp1 targets (Kucinski et al., 2017; Lee et al., 2018). Thus, it is possible that in *Drosophila* p53 itself is not essential for elimination of $Rp^{+/-}$ cells by cell competition because relevant target genes can be activated by Xrp1, bypassing p53 (Fig. 1B).

Interestingly, p53 itself is activated in mammalian cells with *Rp* mutations, such as in patients with Diamond-Blackfan Anemia, one of several ribosomopathies associated with ribosome biogenesis defects, and in *Rp* knockout mouse models. The mammalian $Rp^{+/-}$ cells experience a nucleolar stress in which RpL5 and RpL11, rendered in excess by the reduced rate of ribosome biogenesis in cells with mutations in any of the other ribosomal protein genes, bind to and inhibit MDM2 (in mice; HDM2 in humans), thereby reducing p53 turnover (Deisenroth et al., 2016). No DM2 protein is conserved in *Drosophila*, where p53 turnover is regulated by an unrelated ubiquitin ligase that has not been reported to interact with any ribosomal proteins (Chakraborty et al., 2015), so *Rp* mutations could not activate p53 by this mechanism in *Drosophila*. Instead, the elevated expression of *Xrp1* depends on a different ribosomal protein, RpS12, whose role in cell competition was recently discovered in parallel with that of Xrp1 (Kale et al., 2018). The molecular mechanism of *Xrp1* activation by RpS12 is not known at present. RpL5 and RpL11 are special ribosomal proteins in that they complex with the 5 S rRNA that is transcribed separately from other rRNAs and by RNA polymerase III rather than RNA polymerase I. The resulting 5 S RNP is somewhat stable which facilitates accumulation in the presence of other ribosome biogenesis defects (Donati et al., 2013). By contrast, very little is yet known about the function and regulation of the RpS12 protein, which binds to the 18 S rRNA of the small subunit (Rabl et al., 2011).

These new results suggest that cell competition mechanisms in *Drosophila* and mammals may not be as distinct as may have seemed. The cell interactions that lead to cell competition may depend on pathways that *can* be activated by p53 in both mammals and in *Drosophila,* but whereas they *are* activated by p53 in several mammalian examples, in *Drosophila* $Rp^{+/-}$ cells they are activated by a more downstream transcription factor, by-passing p53 (Fig. 1B). Many questions remain. In the *Drosophila* pathway, how does RpS12 communicate ribosome biogenesis defects to the *Xrp1* gene? How important is Xrp1 for the DNA Damage Response downstream of p53? To what extent does p53 act through downstream factors in mammalian cell competition, where there is an RpS12 protein but no obvious homolog of Xrp1? It would be interesting now to know whether $Rp^{+/-}$ cells are eliminated by cell competition in mammals, as might be expected since differential p53 activity can be a cause of cell competition. $RpL24^{+/-}$ cells are certainly disfavored in chimeras with wild-type cells, but it has not been distinguished whether this simply represents a passive consequence of their differential translation and growth rates, or an additional active process of targeted elimination as is seen in *Drosophila*(Oliver et al., 2004).

While the translation defect of $Rp^{+/-}$ cells in mammals has often been considered independent of the p53 activity (Khajuria et al., 2018),

in fact p53 does affect translation through a variety of mechanisms, including regulation of ribosome biogenesis, regulation of general translation initiation, and regulation of specific mRNAs (Marcel et al., 2015; Harvey and Willis, 2018; Kasteri et al., 2018). In *Drosophila*, Xrp1 reduces the translation rate of $Rp^{+/-}$ cells (Lee et al., 2018). Given the effect of Xrp1 on translation in *Drosophila*, the possible effect of p53, or other regulatory signals activated by ribosome biogenesis defects, on protein synthesis in Diamond-Blackfan Anemia patient cells would bear further investigation.

Cell competition represents an emerging aspect of p53 function that is not cell-autonomous but only apparent between cells with different p53 activity levels. Now it seems that at least one p53-independent example of cell competition may depend on common targets activated by a p53-independent route (Fig. 1B). It may be interesting to determine whether cell competition contributes to the tumor suppressor function of p53 in mammals, which appears not strictly dependent on well-known cell cycle and cell survival targets (Kaiser and Attardi, 2018). It was recently reported that human RpS12 gene dose is frequently reduced in Diffuse Large B-Cell Lymphoma, in a manner mutually exclusive to loss of p53 (Derenzini et al., 2018). In principle this could be consistent with a function of human RpS12 related to that of p53, lthough this remains to be investigated.

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