Blunting senescence boosts liver regeneration

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ABSTRACT

The mammalian liver possesses a unique capacity for regeneration. However, this regenerative potential declines with age due to unknown mechanisms. In this issue of *Genes & Development*, Ritschka and colleagues compare liver regeneration upon partial hepatectomy in young and adult mice. Partial hepatectomy causes a transient increase in p21 in a subpopulation of hepatocytes that persist in adult mice. Remarkably, treatment with the BCL-2 family inhibitor ABT-737 blunts p21 expression enhancing liver regeneration.

MAIN TEXT

Cellular senescence is a stress response that causes a stable cell cycle arrest and is accompanied by distinct changes, including genetic, metabolic and morphological alterations. Senescence prevents the replication of damaged cells. The growth arrest associated with senescence is mediated by induction of the cyclin-dependent kinase (CDK) inhibitors $p21^{Cip1}$ and $p16^{Ink4a}$ (Herranz and Gil 2018). The value of a controlled, well-regulated senescence programme is offset by the accruing evidence that the aberrant accumulation of senescent cells promotes ageing and related disorders. Indeed, a hallmark feature of senescent cells is the enhanced production of soluble signalling molecules; the so-called senescence-associated secretory phenotype (SASP), which mediates many of the consequences of senescence, including those that are detrimental (McHugh and Gil 2018). While parallels between senescence and ageing have long been reported, a causative role for senescence in ageing was provided by the observation that the genetic removal of $p16^{Ink4a}$-positive cells increased lifespan and delayed age-related disorders in mice (Baker et al. 2016). This observation has fuelled research into identifying molecules, termed “senolytics”, that exploit vulnerabilities of senescent cells in order to trigger their demise (Zhu et al. 2015). The therapeutic potential of senolysis has been demonstrated in pathologies as diverse as cardiovascular dysfunction, liver steatosis (Ogrodnik et al. 2017) and neurodegeneration (Bussian et al. 2018).

Senescence has been linked to impaired regeneration following severe liver injury (Krizhanovsky et al. 2008; Bird et al. 2018). However, whether senescence is causally involved in age-associated loss of regenerative capacity in the liver remains unclear. Ritschka and colleagues aimed to unravel the link between senescence and

regenerative decline using partial hepatectomy (PH). PH in adult (6-8-month-old) animals was associated with delayed regeneration and lipid accumulation, in stark contrast to young (2-3-month-old) mice, which showed an almost complete recovery seven days after PH. In young and adult livers, the expression of p21 peaked in a subset of hepatocytes 24-48 hours post-surgery. Interestingly, p21 levels remained aberrantly high in the adult liver. The p21-positive hepatocytes did not express other senescence markers, such as $p16^{Ink4a}$, suggesting that these cells were not fully senescent. Nevertheless, cytokine profiling revealed elevated levels of SASP factors, including IGFBP-3, CCL2 and MMP2 three days post-PH in adult livers. To target the senescence response, researchers administered the senolytic ABT-737 prior to surgery. Compared to vehicle-treated mice, those receiving ABT-737 showed augmented regenerative capacity and improved liver function, evidenced by increased organ weight and normalised serum liver enzymes. Additionally, the lipidosis that emerged following PH in older animals was minimised by ABT-737. At the cellular level, ABT-737 decreased p21 transcript and protein levels, reducing p21-positive hepatocyte burden post-injury. Intriguingly, $p16^{Ink4a}$ was not affected by ABT-737 despite increasing in adult livers pre-hepatectomy, and being a key marker of senescent cells. Indeed, $p16^{Ink4a}$ levels were not increased further following PH and, unlike p21, cells expressing high levels of $p16^{Ink4a}$ were not hepatocytes but macrophages and endothelial cells. Similarly, while a transient increase in expression of the p53 activator $p19^{Arf}$ was observed post-injury, no differences were found between young and adult mice nor did ABT-737 affect $p19^{Arf}$ levels. This highlights the dynamic changes in senescence-associated genes in the immediate aftermath of PH and suggests that the benefits of ABT-737 are likely due to interference with the p21-positive hepatocyte population. This idea is strengthened by the observation that adult p21-deficient mice experienced increased regeneration following PH in the short-term, whereas $p16^{Ink4a}$ and $p19^{Arf}$ deficiency provided no such advantage. Excitingly, a number of SASP-associated cytokines that were elevated in the adult liver post-surgery were dampened by ABT-737, suggesting that the senescence-weakening effects of the compound extend to the associated SASP-like milieu (Figure 1).

The results described by Ritschka and colleagues corroborate past studies demonstrating that hepatocyte and non-parenchymal cell senescence occurs following acute liver damage, leading to impaired regeneration (Krizhanovsky et al. 2008; Bird et al. 2018). An intriguing finding of this study is that the cells most likely to be contributing to defective regeneration in the adult liver, namely hepatocytes that aberrantly express p21, are not yet fully-fledged senescent cells but may represent an

intermediate senescent state. Still, administration of a senolytic compound was able to enhance liver re-growth and function in adulthood. This outcome was attributed to the ability of ABT-737 to modify pre-senescent cellular changes. Indeed, Ritschka et al. failed to detect apoptosis following ABT-737 administration, but instead showed a decrease in the expression of p21 and SASP factors. These findings suggest that a so-called senolytic compound, such as ABT-737, could be exerting its effects at least in part by blunting senescence. How ABT-737 inhibits p21 and associated inflammatory processes in this setting remains to be determined. Regardless of the mechanism, the compelling finding that senotherapy releases the breaks on rejuvenation in adulthood warrants further exploration. This study also emphasises the complexity of the senescence response, exemplified by the dynamic induction of senescence mediators following PH, and by the observation that senolytic treatment did not impact on $p16^{Ink4a}$-positive cells. This contrasts with previous work demonstrating that elimination of $p16^{Ink4a}$-expressing cells ameliorates age- and diet-induced liver steatosis (Ogrodnik et al. 2017). In fact, during PH, $p16^{Ink4a}$ deficiency lead to poorer regeneration. Similarly, the presence of senescent cells has been shown to limit fibrosis following liver injury (Krizhanovsky et al. 2008). The advantages of targeting senescent cells are therefore largely context-dependent, and potentially precarious in nature, given that longer-term deficiency of p21 offered no regenerative edge following PH. This reinforces the notion that senescence modulation must be exceptionally well-regulated and fine-tuned if we are to reap its therapeutic rewards and expand the exciting world of senotherapy further.

COMPETING INTERESTS

J.G. owns equity and has acted as a consultant for Unity Biotechnology and Geras Bio. J.G. is a named inventor in a patent related to senolytic therapies.

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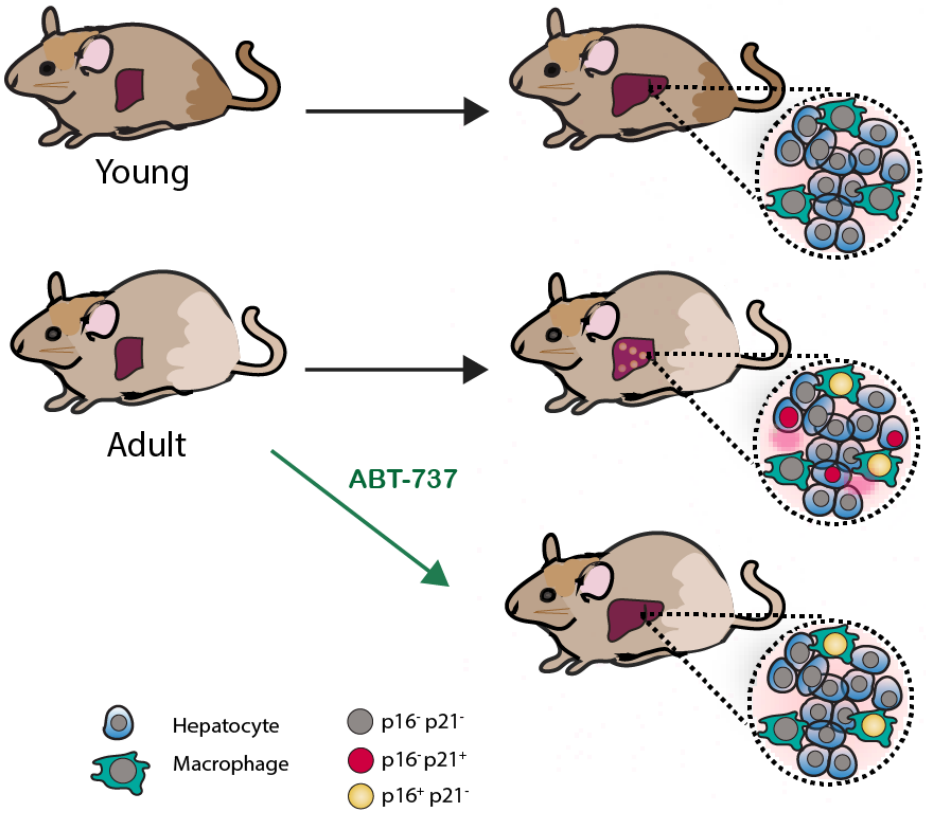

Figure 1. Treatment with ABT-737 improves liver function and promotes regeneration. Adult mice show impaired liver regeneration following partial hepatectomy (PH). A population of p16-positive macrophages is present in the liver of adult mice. Following injury, a subpopulation of hepatocytes expressing aberrant levels of p21 persist in the livers of adult but not young mice. Pre-treatment with the senolytic compound ABT-737 improves liver regeneration and function in adults. ABT-737 inhibits p21 expression in hepatocytes and reduces inflammation without affecting p16-positive liver macrophages.