

COMMENTARY

## RB: An essential player in adult neurogenesis

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### ABSTRACT

The fundamental mechanisms underlying adult neurogenesis remain to be fully clarified. Members of the cell cycle machinery have demonstrated key roles in regulating adult neural stem cell (NSC) quiescence and the size of the adult-born neuronal population. The retinoblastoma protein, Rb, is known to possess CNS-specific requirements that are independent from its classical role as a tumor suppressor. The recent study by Vandenbosch et al. has clarified distinct requirements for Rb during adult neurogenesis, in the restriction of proliferation, as well as long-term adult-born neuronal survival. However, Rb is no longer believed to be the main cell cycle regulator maintaining the quiescence of adult NSCs. Future studies must consider Rb as part of a larger network of regulatory effectors, including the other members of the Rb family, p107 and p130. This will help elucidate the contribution of Rb and other pocket proteins in the context of adult neurogenesis, and define its crucial role in regulating the size and fate of the neurogenic niche.

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It is presently established that neural stem cells (NSCs) reside in the adult mammalian brain: the two discrete neurogenic zones are the subventricular zone (SVZ) lining the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG).<sup>1</sup> These NSCs interact with a complex and tightly coordinated network of intrinsic and extrinsic factors to continuously generate functional neurons throughout life, respectively migrating along the rostral migratory stream (RMS) to the olfactory bulb (OB), or remaining in the DG. In addition to contributing to brain plasticity,<sup>2</sup> experimental augmentation of the adult-born neuron population enhances pattern separation, and may reverse associated impairments that result from normal aging.<sup>3</sup> The therapeutic control of adult neurogenesis offers tremendous potential to enable new approaches in regenerative medicine.<sup>4</sup> However, the search for novel strategies to repair the damaged brain remains a challenge, due to ongoing ambiguity regarding fundamental mechanisms regulating quiescence, activation, commitment and survival.

The orchestration of adult neurogenesis largely relies on two key processes, whose underlying mechanisms remain to be fully elucidated. First is

the ability to maintain a pool of quiescent NSCs (qNSCs), which are sustained throughout life. qNSCs exist independently of the activated NSC subpopulation, which are characterized by upregulated Nestin expression and ultimately enter neurogenesis.<sup>5,6</sup> Maintenance of quiescence is important, as defects such as over-activation can result in the exhaustion and subsequent depletion of qNSCs from the adult neurogenic zones,<sup>7</sup> whereas excessive quiescence ultimately leads to too few differentiated progeny. They are believed to originate embryonically, whether during mid-development for slowly-dividing SVZ qNSCs,<sup>8,9</sup> or late development for SGZ qNSCs, in advance of postnatal SGZ formation<sup>10,11</sup> These quiescent cells undergo reversible transition into an activated state, which is believed to be mediated by factors including *Ascl1*,<sup>12</sup> and BMP signaling specifically in the DG.<sup>13</sup> The second key process is the maintenance of an adult-born neuron population; guiding activated NSCs through self-renewal or commitment to neuronal differentiation, and ensuring their postmitotic survival. The complex regulatory networks directing the fate of activated NSCs have been reviewed elsewhere.<sup>1,14,15</sup> Ultimately, therapies

targeting these processes should enhance the regenerative rate, and expand upon the restricted potential, observed during adult neurogenesis.

### Cell cycle regulation and adult neurogenesis

Several studies have revealed crucial requirements for cell cycle proteins in maintaining both key processes, in coordination with fate determinants and differentiation factors.<sup>16,17</sup> The regulation of NSC quiescence depends on the tight regulation of the cell cycle machinery. CDK-inhibitory protein (CKI) p57<sup>Kip2</sup> is believed to play a key role in the maintenance of quiescence in adult qNSCs. Conditional deletion of p57<sup>Kip2</sup> results in a significant decrease in the embryonically-generated NSC population that goes on to generate the pool of qNSCs in the adult SVZ.<sup>8</sup> Similarly, p57<sup>Kip2</sup> deletion in the adult SGZ results in expansion restricted to the activated NSC population.<sup>18</sup> A recent study further associated the quiescence of SGZ qNSCs to the prevention of cyclin D accumulation, via the rapid degradation of proactivation factor Ascl1.<sup>19</sup> Together, these studies support an intimate link between cell cycle control and adult NSC quiescence.

Maintenance of the adult-born neuron population relies on several cell cycle-related processes guiding the fate of activated NSCs, leading to either self-renewal or differentiation: these include division symmetry, length of the total cell cycle and length of G1 phase specifically.<sup>20-22</sup> Mechanistically, Cdk6 - and not Cdk4 - has been shown to distinctly mediate the expansion of differentiating adult-born progenitors,<sup>22</sup> while mice deficient for cyclin D2 lack adult-born neurons.<sup>23</sup> Furthermore, Cdk4-cyclinD1 complex overexpression in the SGZ induces expansion of the activated NSC and progenitor populations at the expense of differentiation.<sup>24</sup>

CKIs p21<sup>Waf1</sup> and p27<sup>Kip1</sup> have each demonstrated regulatory roles mediating both of these key processes. p21<sup>Waf1</sup> is required for adult NSC quiescence, as deletion in the SVZ leads to expansion and reduced longevity of the self-renewing NSC population,<sup>7</sup> concurrent with premature terminal differentiation through expression of Bmp2<sup>25</sup> Furthermore, separate studies in the SVZ and SGZ respectively demonstrated p21<sup>Waf1</sup> repressing NSC self-renewal through the direct binding and repression of Sox2<sup>26</sup> at its downstream SRR2 enhancer, and suppressing proliferation

specifically in differentiating adult-born neurons.<sup>27</sup> p27<sup>Kip1</sup> is upregulated in the SGZ by pro-quiescence factor Bmp4, preventing expansion of the self-renewing NSC population.<sup>28</sup> p27<sup>Kip1</sup> further suppresses proliferation specifically in differentiating SVZ progenitors.<sup>29</sup> Adult mice null for p27<sup>Kip1</sup> demonstrate significantly increased basal levels of Sox2 expression in their brain tissue, suggesting that p27<sup>Kip1</sup> may also be involved in repressing Sox2 in the CNS,<sup>30</sup> with similar implications for NSC self-renewal. Together, these studies support an important link between cell cycle control and adult neurogenesis.

The retinoblastoma protein (Rb) operates at the core of the canonical cell cycle pathway, representing a point of convergence for cyclin, Cdk and CKI activity. Typically classified as a tumor suppressor, Rb and Rb-like proteins p107 and p130 form the Rb family of pocket proteins, named for the homologous A/B binding pocket domain each uses to regulate E2F transcription factors.<sup>31</sup> Rb proteins regulate cell cycle progression at the G1/S restriction point. In response to mitogenic stimuli, the Cdk4/6-cyclin D complex progressively phosphorylates/inactivates Rb, resulting in the release of E2Fs, whose transcription promotes entry into S phase.<sup>32,33</sup> Rb has a demonstrated requirement in maintenance of quiescence, as Rb deletion is sufficient to induce cell cycle re-entry in several systems,<sup>31</sup> including MEFs,<sup>34</sup> mammalian muscle cells,<sup>35,36</sup> and adult cortical neurons.<sup>37</sup> It has also been implicated in the regulation of iPSC self-renewal, as Rb inhibits Sox2 by repressing its downstream SRR2 enhancer.<sup>38</sup> While these studies have directly implicated Rb in maintaining both key processes, the requirement of Rb specifically during adult neurogenesis remains to be clarified.

### Rb and embryonic CNS development

Previous studies have demonstrated distinct requirements for Rb during embryonic development, which go beyond its traditional role in regulating the cell cycle machinery.<sup>39</sup> These requirements are relevant to adult neurogenesis, as recent findings suggest quiescent adult NSCs are derived from cell populations born during embryonic development.<sup>8-10</sup> During cortical development, studies have demonstrated a requirement for Rb in restricting proliferation in committed neuroblasts.<sup>40</sup> As conditional Rb-deficiency did not result in widespread apoptosis, this initial result

suggested that Rb loss might be used to experimentally augment neurogenesis. Studies demonstrated further requirements for Rb in differentiation of the ventral telencephalon, by preventing the E2f-mediated direct suppression of *Dlx2*,<sup>41</sup> as well as in tangential migration into the neocortex,<sup>42</sup> regulating the expression of netrin/RGM receptor neogenin.<sup>43</sup>

A recent study by Vandenbosch et al.<sup>44</sup> suggests a key requirement for Rb during hippocampal development, and the generation of dentate granule cell neurons (DGCs). While Rb deletion in developing NSCs demonstrated no effects on self-renewal, it led to enhanced DGC neurogenesis, including a dramatic 3-fold increase in proliferating cell number and a significantly expanded DGC population. Rb deletion further led to a small yet significant increase in apoptosis. While there was an ultimate net gain to the DGC population, this may reflect a compensatory mechanism to reduce the augmented DGC population, or a potential requirement for Rb for DGC survival during development.

### Rb and adult neurogenesis

While little is known regarding the role of Rb during adult neurogenesis, the use of Tamoxifen-inducible transgenic mouse models have helped overcome the embryonic lethality of Rb deficiency.<sup>45</sup> Naser et al. recently demonstrated roles for Rb in adult SVZ/OB neurogenesis both consistent with, and distinct from, those observed during development.<sup>46</sup> Consistent with development, Rb does not appear to regulate self-renewal of adult NSCs, and is required to control progenitor proliferation in the adult SVZ and RMS. Distinctly, the authors were not able to detect a role for Rb in maintaining NSC quiescence, and Rb-deficient nascent neurons did not demonstrate any apparent defects in differentiation or rostral migration. While Rb loss led to an increase in OB neurogenesis, it was abrogated one month later by increased apoptosis. This suggests a requirement for Rb in the long-term survival of adult-born OB neurons.

Interestingly, a recent study by Vandenbosch et al.<sup>44</sup> further revealed distinct requirements during adult hippocampal neurogenesis. In the adult DG, Rb is required to regulate the cell proliferation of immature newborn DGCs, and to maintain their capacity to produce mature DGCs. Moreover, Rb is essential for the survival of adult-born DGCs, as Rb loss results in

massive DGC death. No evidence for a role for Rb in maintaining NSC quiescence in the adult SGZ was detected in these studies.

Unique to the hippocampus, this study demonstrates that some Rb requirements may be functionally conserved between embryonic development and adult neurogenesis, including regulating proliferation within immature DGCs, and potentially in ensuring DGC survival. Notably, while Rb loss results in expansion of the developing DG, the modest increase in ectopic proliferation does not result in an expansion of the DGC population in the adult DG. This suggests the presence of other effectors in regulating the adult-born DGC population size.

This study further defines a crucial requirement for Rb in the short term survival of adult-born DGCs, in contrast to its requirement during adult SVZ/OB neurogenesis, where Rb loss transiently expands the number of neurons populating the OB. These results support the hypothesis that regional differences distinguish the NSCs of each neurogenic niche,<sup>47</sup> which may be regulated by relatively dissimilar transcriptional programs and indicate distinct cell type-specific roles for Rb. Nonetheless, these results demonstrate two roles for Rb conserved between adult neurogenic zones: restriction of proliferation, and long-term survival.

### Putting Rb in context

Although Rb demonstrates important roles in the regulation of NSC and neuronal populations during brain development, its function during adult neurogenesis appears to integrate the contributions of other regulatory mechanisms. The study by Vandenbosch et al.,<sup>44</sup> together with the recent study by Naser et al.,<sup>46</sup> do not reveal any cell cycle-dependent effects on uncommitted NSCs from either neurogenic niche, in response to Rb deletion. This suggests that Rb is dispensable in maintaining the quiescence of adult NSCs. While this is consistent with prior findings in adult haematopoietic stem cells,<sup>48</sup> this notably suggests that Rb may not serve as the main cell cycle regulator governing adult NSCs. Furthermore, the results from Vandenbosch et al. suggest the presence of other effectors specifically regulating the adult-born DGC population size, with intrinsic implications for adult-born neurons originating from the SVZ. This prompts the question: if Rb does not independently regulate adult

NSC quiescence and modestly regulates the size of the adult-born neuronal population, what does?

Shifting from the contribution of Rb alone toward a broader regulatory network, we must consider the involvement of the other pocket proteins, p107 and p130. The shared pocket domain results in some functional overlap between members, particularly through the E2F family of transcription factors.<sup>49-51</sup> Inactivation of all three Rb family members demonstrates distinct requirements from Rb inactivation alone,<sup>31</sup> as demonstrated during the regulation of quiescence and proliferation in haematopoietic<sup>52</sup> and liver<sup>53</sup> systems.

Prior studies have identified key regulatory requirements for p107 during adult neurogenesis. Relating to maintenance of quiescence, p107 is expressed only in uncommitted NSCs and progenitors within the SVZ, and acts in dual roles: the suppression of self-renewal by downregulating the Notch pathway through direct Hes1 repression, as well as the favoring of neuronal differentiation and commitment.<sup>54,55</sup> While the quiescence-specific role for p107 remains to be clarified, studies have since identified the role of high Notch activity<sup>56</sup> in promoting NSC quiescence. p107 has been further implicated in the maintenance of adult-born neuronal population size. E2F3 isoforms E2F3a and E2F3b, bound by p107, hold opposing roles in directly targeting Sox2 at its proximal promoter region, as demonstrated in activated NSCs.<sup>57</sup> This demonstrated the intimate involvement of p107 in the cell cycle-independent regulation of NSC self-renewal.

The contribution of p130 to adult neurogenesis remains the least established among Rb family members. During iPSC differentiation, p130 forms a repressive complex with E2F4 and SIN3A to repress Sox2 expression via its SRR2 enhancer, potentially in synergy with similar repression by p27<sup>Kip1</sup>.<sup>30</sup> While this mechanism has yet to be established in the CNS, it may suggest a potential cell cycle-independent role for p130 in regulating NSC differentiation. Moreover, p130 has been implicated in the regulation of cortical neuronal death and survival through E2F4-mediated repression of pro-apoptotic genes,<sup>58</sup> suggesting a potential role for p130 in adult-born neuronal survival. Therefore, it is likely that the true requirements for Rb during adult neurogenesis are masked by functional compensation.

## Conclusion

Investigating the mechanisms by which adult NSCs maintain quiescence and regulate the size of the mature neuronal population is essential for our understanding of neurogenesis. The recent study by Vandenbosch et al.<sup>44</sup> has demonstrated a clear requirement for Rb during adult hippocampal neurogenesis, regulating the production of newborn neurons and ensuring their survival. It further demonstrates that unlike in other systems, Rb does not play a crucial role in the maintenance of NSC quiescence during adult neurogenesis. Future studies accounting for the functional compensation of Rb family members p107 and p130 will help clarify the requirement of Rb in the adult, and contribute to the future development of regeneration therapies to repair the damaged brain.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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