NEURAL REGENERATION RESEARCH
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New pharmacological tools: the use of diterpenes to promote adult hippocampal neurogenesis

Ricardo Gómez-Oliva, Pedro Nunez-Abades, Carmen Castro*

Tissue regeneration maintains homeostasis and preserves the functional features of each tissue. However, not all tissues show a strong repairing capacity. This is the case of the central nervous system. It is now well established that the generation of new functional neurons from stem cells in the adult brain occurs in specific regions of the brain of different species such as rodents, birds, primates, and humans (Eriksson et al., 1998). The brain areas in which neurogenesis occurs are referred to as neurogenic niches, regions in which neural stem cells (NSC) are surrounded by cells and signaling molecules that lead their fate toward mature neurons. Two main neurogenic niches have been thoroughly described in adult rodents: the subventricular zone and the dentate gyrus of the hippocampus (DG). NSC in the adult DG remain mainly in a quiescent non-proliferative state (qNSC) and are able to generate neurons through a complex hierarchical process that involves the activation of neural stem cells, the generation of undifferentiated progenitors, and the origination of immature neurons that differentiate into mature functional neurons. Newly generated neurons migrate short distances from the subgranular zone to the granular layer, where through their axons, they integrate into pre-existing circuits by establishing connections with CA3 and CA2 regions. These neurons participate in several tasks that involve the hippocampus such as memory consolidation, spatial and temporal pattern discrimination, or forgetting, among others.

Aging is a major negative regulator of adult hippocampal neurogenesis in several mammalian species, including non-human primates. Hippocampal neurogenesis declines sharply with age while gliogenesis (astrocyte generation) increases. The maintenance of a neurogenic rate is possible because of the existence of homeostatic mechanisms that preserve the neurogenic pool of NSC in the old DG (Martín-Suarez and Encinas, 2021). Particularly, this preservation is achieved in the DG by maintaining NSC as quiescent non-dividing cells that produce a low number of neurons. The homeostatic mechanisms that preserve NSC are altered by neurological disorders that promote the activation of NSC generating astrocytes and aberrant neurons and depleting the pool (Diaz-Moreno et al., 2018). Studies of human brains show that aging accompanied by progressive hippocampal degeneration, is concomitant with a negative regulation of adult hippocampal neurogenesis (Moreno-Jimenez et al., 2019; Terreros-Roncal et al., 2021). A correlation between the loss of immature neurons and cognitive decline has been shown to occur in aged adults. In light of this finding, it may be reasonable to hypothesize that promotion of neurogenesis may foster new therapeutic possibilities for the aging brain. Preventing the alteration of neurogenesis induced by neurological disorders may help prevent the cognitive impairment associated with these disorders.

Target molecules to promote adult hippocampal neurogenesis in the aging brain: Several molecules have been studied that modulate adult hippocampal neurogenesis in the aged rodent brain. Most of these molecules participate in the maintenance of the NSC pool and determine NSC fate toward a neuronal or glial phenotype within the niche.

Key molecules involved in maintaining neurogenesis in the aged DG include the bone morphogenetic proteins (BMP2 and BMP4). These signaling molecules through their type 1 receptor BMPR1A participate in adult hippocampal neurogenesis regulating the balance between NSC quiescence and proliferation, thus maintaining the stem cell activity required to continuously generate neurons in the mature DG. The activation of the BMP-initiated canonical signaling cascades facilitates differentiation toward a neuronal fate-reducing oligodendrogenesis. To exert this effect, the BMP signaling pathway synergistically interacts with the canonical Wnt signaling pathway. Interestingly, neuropathological aging dramatically increases the expression of BMP6 in the DG contributing to the depletion of the adult hippocampal NSC due to their BMP-mediated differentiation into astroglial cells. In this context, BMP antagonists such as Noggin have been proposed as pharmacological tools to rescue stem cell depletion and to promote neurogenesis during pathological aging (Diaz-Moreno et al., 2018).

Different components of the Wnt signaling cascade are expressed in the adult hippocampus and participate in the regulation of adult DG neurogenesis. Several findings have led to the idea that ligands that initiate this signaling pathway (Wnt1, Wnt3, or Wnt7) and their receptors play a role in regulating the balance of qNSC/activated neural stem cells as well as in determining differentiation of progenitors towards a neuronal fate. Wnt ligands are produced by NSC and astrocytes in the DG neurogenic niche. Several reports show that NSC respond to Wnt-βcatenin signaling (canonical pathway) promoting both NSC self-renewal and proliferation of undifferentiated progenitors and show that the Wnt inhibitors produced in the DG by granule neurons regulate NSC activity rate. Similarly, non-canonical Wnt signaling initiated by Wnt5 plays a neurogenic role in the adult hippocampus (Arredondo et al., 2020b).

One of the homeostatic mechanisms affected in the aged brain, that might contribute to the decline in neurogenesis is the Wnt signaling pathway. A downregulation of Wnt ligands and an upregulation of Wnt inhibitors (Dkk1 or sFRP3) has been observed in the aged brain that impairs neurogenesis (Urban et al., 2019; Arredondo et al., 2020a) and the Wnt-βcatenin signaling has been proposed as a potential therapeutic target for neurodegenerative diseases. In light of these observations, strategies to promote the regulation of the Wnt signaling cascade have been proposed as useful tools for the promotion of hippocampal neuron replacement in the aged hippocampus (Arredondo et al., 2020a; Gomez-Oliva et al., 2020).

In addition to the pathways mentioned above, the aged brain has been shown to respond to exogenous growth factors increasing neurogenesis. In healthy young rodents, astrocytes provide immature neurons with fibroblast growth factor-2, however, in aged animals, reactive astrocytes produce less fibroblast growth factor-2, which is a key factor in neuronal maturation. The use of exogenous fibroblast growth factor-2 has been proposed as a potential therapeutic tool to promote neurogenesis and improve cognition in age-associated neurological disorders.

Likewise, Notch is another signal with the capacity to regulate adult neurogenesis, which is altered in the aged brain. Notch-responsive NSC become quiescent during aging and stop producing new neurons. Notch signaling stimulation may revert this alteration promoting neurogenesis.

New strategies to promote neurogenesis and prevent cognitive impairment in the aged brain–the use of diterpenes: In previous paragraphs, we have mentioned pathways that are being explored to promote neurogenesis in the aging brain. However, the purpose of this article is to highlight the role of new molecules that have been recently proposed as useful tools for promoting neurogenesis and preventing cognitive impairment in the aging brain. These molecules stimulate the epidermal growth factor receptor (EGFR) and its associated pathways. In the DG, NSC expresses EGFR although in a lower proportion in comparison with NSC and progenitors of the subventricular zone. EGFR plays a role in determining the activation, proliferation, and expansion of NSC within the DG (Guo et al., 2022). In addition, its interaction with other pathways, such as the Notch signaling pathway, regulates NSC number and self-renewal. The use of molecules that activate NSC through the EGFR signaling cascade may be useful when designing strategies to promote neurogenesis and prevent cognitive impairment in the aged brain.

Diterpenes with 12-deoxyphorbol structure were described as potent non-tumor promoting protein kinase C (PKC) activating molecules that induce neurogenesis *in vitro* and *in vivo*. They stimulated the proliferation of NSC *in vitro* and the generation of neurons in both the subventricular zone and DG *in vivo* (Geribaldi-Doldan et al., 2015). The study of their mechanism of action revealed that, by activating PKCα, they facilitate the release of EGFR ligands via their phosphorylation (**Figure 1**). Phosphorylated EGFR pro-ligands are selected by ADAM17 for their soluble extracellular n-terminal ligand shedding (Geribaldi-Doldan et al., 2019; Dominguez-Garcia et al., 2021), thus facilitating EGFR ligand release (**Figure 1**). The activation of EGFR activate the MAPK signaling cascades and stimulate the expression of cyclins.

Recent evidence shows that the intracerebroventricular infusion of small-molecule diterpene-based drugs enhances adult neurogenesis in the DG (Dominguez-Garcia et al., 2021). Particularly, the work of Gomez-Oliva et al. (2023) describes that the use of diterpenes with a 12-deoxyphorbol structure, which stimulate neurogenesis in the brain of a murine model of neuropathological aging prevents cognitive impairment, and leads to the production of fully mature neurons. To perform this study, the authors use the SAMP8 murine model of accelerated aging. These mice recapitulate the transition from healthy aging to Alzheimer's disease and show cognitive deficits as well as Alzheimer's disease-like phenotype starting at 6 months of age. In these mice, neurogenesis is stimulated as a consequence of the aging process as soon as 2 months after birth (Diaz-Moreno et al., 2018). This response declines in older mice in

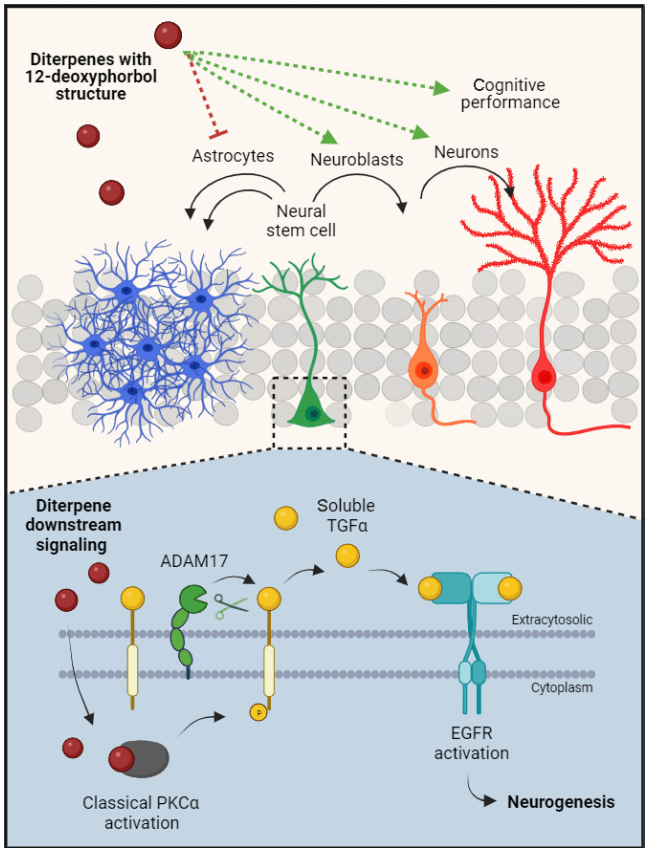

Figure 1 | **Effect of diterpenes with 12-deoxyphorbol structure on neurogenesis in the aged brain.**
According to Gomez-Oliva et al. (2023), diterpenes with 12-deoxyphorbol structure (dark red spheres) activate classical PKC allowing the release of EGFR signaling molecules (yellow spheres) from their pro-ligands. The activation of EGFR-initiated pathways will stimulate not only neural stem cell activation, but also it will lead the neural stem cell fate into newly generated functional neurons, avoiding astrocytosis and facilitating cognitive performance. Created with BioRender.com. ADAM17: A disintegrin and metalloprotease 17; EGFR: epidermal growth factor receptor; PKCα: protein kinase C alpha; TGFα: transforming growth factor alpha.

which newly generated neuroblasts and neurons are aberrantly misplaced within the different layers of the DG (Diaz-Moreno et al., 2018). The work of Gomez-Oliva et al. (2023) demonstrates that the treatment of SAMP8 mice with the diterpene with 12-deoxyphorbol structure ER272, from the age of 4 months to 6 months, prevents the cognitive decline characteristic of 6-month-old SAMP8 mice and stimulates neurogenesis, leading to the generation of fully differentiated non-aberrant neurons within the DG. Interestingly, the increase in neurogenesis observed in treated mice, is a consequence of the activation of qNSC within the DG, that leads to a higher number of neuroblasts and neurons and a reduction in the proportion of mature astrocytes. As a consequence of the activation of NSC, the treatment partially reduces the number of qNSC although it does not result in the exhaustion of the NSC pool.

As a conclusion, in situations in which degeneration is progressing, as it may occur in the damaged aged brain, a remarkable need to compensate for neuronal loss exists. However, the homeostatic mechanisms that regulate neurogenesis are altered and it is not possible to reach higher neurogenic rates that may compensate neuronal loss and prevent cognitive impairment. In this scenario, as depicted in **Figure 1**, diterpenes with 12-deoxyphorbol structure might work at stimulating NSC activation and leading their fate towards functional neurons to avoid a cognitive decline in the aged neuropathological brain by stimulating neurogenesis.

This work was supported by the Spanish Ministerio de Ciencia, Innovación y Universidades (grant number RTI-2018-099908-B-C21 and RTI-2018-099908-B-C22 granted to CC), by the Consejería de Economía, Conocimiento, Empresas y Universidades (grant number FEDER-UCA18-106647 granted to CC) and by the Consejería de Salud y Familias 80% co-financed by EDRF ITI regional funds (ITI-Cadiz-0042-2019 to CC).

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Date of submission: July 31, 2023
Date of decision: October 9, 2023
Date of acceptance: October 25, 2023
Date of web publication: December 11, 2023

https://doi.org/10.4103/1673-5374.389635
How to cite this article: *Gómez-Oliva R, Nunez-Abades P, Castro C (2024) New pharmacological tools: the use of diterpenes to promote adult hippocampal neurogenesis. Neural Regen Res 19(8):1629-1630.*

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C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y