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An "Orphan" Finds a Home in NSC Regulation

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Small molecules that can alter stem cell fate are of immense biological and therapeutic values. In this issue of *Chemistry & Biology*, Saxe and colleagues [1] report a chemical genetic screen that identified an orphan ligand, P-Ser, which can modulate neural stem/progenitor cell fate.

Neural stem cells (NSCs) are multipotent cells that have the capacity to self-renew and have the potential to give rise to all the three fundamental cell lineages of the central nervous system. Unlike many other tissue stem cells, NSCs differ in their multipotency and lineage commitment depending on the developmental stage of the organism as well as the region of the brain from which they are isolated [2]. However, considering the ethical and political debates surrounding the use of human embryonic stem cells, NSCs offer a noncontroversial alternative to developing stem-cell-based therapies for many incurable neurodegenerative diseases as well as in exploring novel therapies for brain cancer. Moreover, the recent finding that multipotent stem/progenitor cells exist at least in three distinct regions of the adult mammalian brain, where neurogenesis persists through adulthood [3], has elicited a genuine possibility of noninvasive endogenous brain repair as a viable alternative for cell transplantation therapy. However to exploit the full therapeutic potential of NSCs it is critical to have an in-depth understanding of the molecular mechanisms underlying their self-renewal, differentiation, migration, and survival. Additionally, reagents and methods need to be generated that could provide a continuous supply of these cells and manipulate their cell fate choice.

Neural stem/progenitor cells can be isolated from embryonic, fetal, or adult brain tissues and after dissociation they can be expanded in vitro as monolayer or as floating spheres of cells in the presence of growth factors such as bFGF and/or EGF. Such neurospheres contain a heterogeneous mix of stem cells, progenitors, and differentiated cells. Growth factor responsive stem/progenitor cells from such a mixture can be serially passaged to form new spheres and can be expanded over at least several passages [4]. However, their proliferative and multipotent nature drastically declines over several passages. Upon growth factor withdrawal NSCs spontaneously differentiate, to a lesser extent to neurons and mostly to astroglia. Though having its own technical shortcomings, neurosphere assay is still one of the most widely used techniques for isolating and validating neural stem cells from embryonic and adult CNS.

Candidate gene approaches and knockout animal models have produced vital information regarding the biology of NSCs. However, such studies so far are restricted mostly to highly studied pathways such as Wnt, Shh, and Notch, etc. Considering the wealth of genetic information available to us in the postgenomic era, and the complexity of gene and protein interaction networks that operate in concert with cell extrinsic cues to produce a given phenotype, such gene-centric approaches could yield only limited information in a given period of time. Cell-based high-throughput phenotypic screens using cDNA and RNAi arrays have recently emerged as a technique for whole genome level studies. Synthetic small molecule libraries offer a simple cost-effective, yet highly valuable, alternative to this approach [5].

Historically, small molecules have contributed immensely to the progress in biological science. Small molecules have been identified that are capable of modulating specific stem cell fate (e.g., all-trans retinoic acid, ESCs, NSCs, MSCs) and some have found several clinical applications (e.g., Gleevac-imatinin mesylate; cancer stem cells in leukemia). Since there are endless possibilities of generating structurally diverse small molecules, this approach has been used to target an increasing number of proteins. Using advanced automation combined with chemiluminescence/fluorescence microscopy or image-based detection for end-point read out (appropriate marker staining or reporter gene activity and/or cell morphology), millions of small molecules can be screened for a phenotype in a short period of time.

Though extremely powerful there are only few studies reported to date that have employed the aforementioned strategy to study NSCs or stem cells in general. Using the above approach, we reported a novel small molecule (from a combinatorial library of 50,000 compounds), neuropathiazol, which selectively induced robust differentiation of multipotent hippocampal neural progenitors to neurons [6]. More recently Diamandis and colleagues, using a similar strategy, screened a library of 1267 pharmacologically active compounds for inhibitors of neurosphere proliferation [7]. The screen results revealed a complex interplay of signaling pathways that govern the proliferation and self-renewal of NSCs. Interestingly many of these compounds, which are modulators of adult neurotransmission pathways, have antiproliferative effects on brain cancer stem cells, too.

In this issue of *Chemistry & Biology*, Saxe et al. [1] describe an elegant study that reports the identification of

inhibitors and enhancers of neuronal differentiation of mammalian NSCs, by screening hundreds of bioactive compounds. Following up on one of those enhancers, the orphan ligand phosphoserine (P-Ser), the authors demonstrate a novel link between the group III metabotropic glutamate receptor 4 (mGluR4; yet another neurotransmission component) and neurogenesis.

In the initial screen using neurospheres generated from embryonic day 11.5 (E11.5) brain the authors identified 64 hits (a surprisingly high number considering the total number of compounds screened) from which fourteen were selected for secondary assays. Five compounds from that group were confirmed to modulate neuronal differentiation (four enhancers and one inhibitor). Clonal analysis and RT-PCR for neurogenic transcription factors revealed that P-Ser treated cultures were twice as neurogenic as the control. Furthermore, time course experiment and TUNEL assay of E14.5 neuronal cultures suggested a neuroprotective effect of this compound.

Target identification and elucidation of the mechanism of action is often the most challenging aspect of chemical genetic screens. However, in the existing literature there are indirect evidences that connect P-Ser to neurogenesis. In the brain P-Ser acts as the substrate for phosphoserine phosphatase (PSP) that converts it to L-serine, an essential amino acid that has a critical role in cellular proliferation, cell–cell communication, and as a glial-derived trophic factor. Recently, PSP was identified to be specifically expressed by the neural progenitors and stem cells within the neurogenic region in adult and embryonic brain [8]. Additionally, PSP knockdown could inhibit proliferation of the neural progenitors, indirectly suggesting that unmetabolized P-Ser may contribute to this phenotype.

Interestingly, it is observed that P-Ser can specifically bind to the glutamate receptor mGluR4 with very high affinity [9]. Enhancers of mGluR4 are also reported to have an inhibitory effect on the proliferation and promote the differentiation in cerebellar granule cell neural precursors [10]. However a direct role of this receptor in neuronal differentiation of NSCs is not established. The authors hence tested the involvement of mGluR4 in the observed phenotype. To their delight, inhibition of mGluR4 by siRNA or through its specific antagonist abrogated the neurogenic and antiproliferative activity of P-Ser, thus strongly suggesting that its observed activity is mediated through mGluR4.

Group III metabotropic receptors function by reducing the cAMP levels through inhibition of Gs activated adenylyl cyclase [8]. However forskolin, an adenylyl cyclase activator, has been found to induce neurogenesis in P19 embryonal carcinoma cells, ES-cell-derived neuroepithelial cells, and rodent neural stem cells [5]. Thus it is intriguing that the inhibitor of such activity can also elicit the similar phenotype in NSCs. It is possible that neurogenic activity of mGluR4 may be independent of its action on cAMP levels or this activity may be restricted to stage-specific subsets of progenitors. Considering the mosaic organization of NSCs in adult mammalian brain [2], it would be interesting to test whether P-Ser has similar neurogenic activity in diverse population of NSCs. Additionally, usefulness of P-Ser in inhibiting the proliferation of cancer stem cells could also be explored. Pharmacological testing of P-Ser and other mGluR4 agonists in appropriate animal models would help determine the therapeutic potential of those compounds.

In summary, Saxe et al. [1] have described a simple chemical genetic screen to identify modulators of neuronal differentiation of neural stem/progenitor cells. The methodology can be conceptually applied for the study of other stem cells. Their research has also opened up new avenues that can be explored for therapeutic interventions of neurodegenerative disorders and studying the biology of neural stem cells.

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