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Visions & Reflections

Insulin/IGF signalling in neurogenesis

J. M. Bateman [a, *] and H. McNeill [b]

[a] The Wolfson Centre For Age-Related Disease, Hodgkin Building, King's College London, Guy's Campus, London, SE1 1UL (United Kingdom), Fax: +44 207 848 6816, e-mail: joseph_matthew.bateman@kcl.ac.uk
[b] Samuel Lunenfeld Research Institute, 600 University Avenue, Room 884, Toronto, Ontario M5G 1X5 (Canada)

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Introduction

A fundamental challenge during the development of any complex organism is the coordination of proliferation and differentiation. In the case of neurogenesis, cells must exit the cell cycle and undergo a complex programme of gene expression and morphological changes. This requires the action of multiple secreted ligands which, by binding to their target receptors on the cell surface, control the course of neuronal cell fate in a spatiotemporal manner. Neurogenic organs are wholly dependent on prior proliferation to provide enough cells to generate the mature tissue. There are often assumed to be two sets of independent signalling pathways: one which controls proliferation and a second which controls differentiation. In this context, neuronal differentiation might be seen as a default pathway that occurs as a result of growth factor removal. Surprisingly, however, the same pathway often regulates both proliferation *and* differentiation. In this review we discuss the role of the insulin receptor (IR) and the type I insulin-like growth factor receptor (IGF-IR) receptor tyrosine kinases (RTKs) in neuronal differentiation by comparing knowledge about vertebrates with insight gained from studies in *Drosophila*. Evidence from vertebrates and flies suggests that, in certain developmental contexts and cell types, IR/IGF-IR signalling plays an important role in the differentiation of neurons.

* Corresponding author.

Insulin/IGF signalling in vertebrate neurogenesis

Although the role of IR and IGF-IR signalling in cell proliferation has been clearly demonstrated, the potential role of this group of RTKs in neuronal differentiation has received less attention. Insulin is best known for its role in glucose uptake and metabolism, whereas the insulin-like growth factors (IGFs) are well characterised as growth-promoting peptides [1]. Expression studies of the IR and IGF-IR have demonstrated that both of these RTKs are expressed in the nervous system [2, 3], suggesting that they function in neuronal development. The IR is widely expressed throughout the adult brain and concentrated expression is found in the hypothalamus, olfactory bulb and pituitary [3–5]. In addition, the IGF-IR is expressed in many embryonic tissues but high levels of expression are seen in the developing cerebellum, midbrain, olfactory bulb and the ventral floorplate of the hindbrain [2].
In cultured cells, insulin and IGF-I do not always act as mitogens. For example, in mouse fibroblast cell lines, insulin and IGF-I are very poor mitogens [6]. Insulin and IGF-I can also activate neurogenesis in ex vivo and cultured cell lines [6–11]. H19-7 rat hippocampal cells proliferate at 34 °C in response to serum and differentiate to a neuronal phenotype at 39 °C when treated with basic fibroblast growth factor (bFGF). However, expression of the IGF-IR allows HC19-7 cells to differentiate at 39 °C in response to IGF-I independent of bFGF [9]. In E14 mouse striatal primary neural stem cells (NSCs), the action of insulin/IGF-I to activate either proliferation

or differentiation is dependent on the passage number of the cells. NSCs isolated from neurospheres after two rounds of culture for 1 week differentiate to a neuronal phenotype in response to treatment with IGF-I [7]. Interestingly, the neurogenic action of IGF-I could be potentiated by the addition of brain-derived neurotrophic factor (BDNF), suggesting that these factors can act synergistically to promote differentiation. Conversely, treatment of similar NSCs from primary cultures with IGF-I caused individual cells to proliferate rapidly rather than differentiate [8]. Therefore, the ability of insulin/IGF to promote either differentiation or proliferation depends on the cell type and conditions.

What do the phenotypes of *Ir* and *Igf1r* mutant animals tell us about the role of these RTKs in neurogenesis? *Ir–/–* null mice develop normally but die shortly after birth due to severe diabetic ketoacidosis [12], suggesting that the IR is not required for neuronal development. Moreover, a neuron-specific disruption of the *Ir* gene in mice did not affect brain development or neuronal survival [13]. In contrast, *Igf1r–/–* mice have reduced brain size and altered brain structures, including a marked increase in the density of neural cells in the spinal cord and brainstem [14]. Furthermore, detailed examination of cochlear development has shown that development of this sensory organ is severely impaired in *Igf1r–/–* mice [15]. A significant decrease in the number of auditory neurons along with aberrant expression of early neural markers suggests that neuronal differentiation in the inner ear is delayed in these mice. Recent studies have also shown that IGF-I is required for differentiation of neuroblasts in the otic vesicle in chick [16]. Moreover, differentiation of neurons derived from mouse olfactory bulb stem cells requires IGF-I [17]. Thus, in mice the IGF-IR seems to be essential for correct central nervous system (CNS) development, while the IR may either be redundant or play a more subtle role.

What are the intracellular signalling cascades by which the IR and IGF-IR RTKs have the potential to control differentiation? In mammalian systems, insulin stimulation has been shown to cause activation of the Ras/mitogen-activated protein kinase (MAPK) pathway [18–20]. Activation of MAPK by the IR is independent of the role of this receptor in glucose homeostasis since inhibition of MAPK activation does not affect the metabolic actions of insulin [21]. Ligand binding to the IR results in tyrosine phosphorylation of the insulin receptor substrate (IRS) proteins and/or Shc, which, through the adaptor protein Grb2, results in recruitment to the membrane of SOS for the activation of Ras (Fig. 1) [22, 23]. MAPK activation is the most well defined route by which IR/IGF-IR signalling might control neurogenesis during development. The first *in vivo* evidence for insulin stimulation of Ras came from the demonstration that insulin-induced *Xenopus* oocyte maturation is blocked by an antibody which inhibits Ras [24]. More recently, knock-out mice studies have shown that MAPK activation by insulin *in vivo* is dependent on IRS-1 [25]. In cultured cells, activation of MAPK is required for nerve growth factor (NGF)/epidermal growth factor (EGF)-dependent differentiation of PC12 cells [26]. Activation of MAPK in PC12 cells causes phosphorylation of target transcription factors and consequent reprogramming of gene expression to a neuronal fate [27]. Activation of MAPK by an IR/IGF-IR receptor-dependent mechanism has the potential to activate a similar neurogenic switch in target cells in the developing nervous system.

The other pathway which is activated by insulin/IGF receptor stimulation is PI3K/TOR signalling (Fig. 1). PI3K/TOR kinase signalling is known to regulate growth through the control of ribosome biogenesis and protein synthesis [28]. PI3K catalyses the conversion of PIP2 to PIP3, a process which is reversed by the lipid phosphatase PTEN. Growth control is mediated through TOR by the activation of S6K and the translation initiation factor eIF4E. The possible role of PTEN in the nervous system has been studied by several groups using conditional knock-out strategies. Although PTEN is not essential for cell fate determination in the CNS overall [29, 30], a dramatic effect was observed in glial cells. Yue et al. [31] used GFAP-cre to generate *pten–/–* cells in the CNS and observed premature differentiation of Bergmann glia in the early postnatal brain. The premature differentiation of *pten–/–* glia resulted in layering defects and subsequent aberrant migration of granule neurons. These data support a role for PTEN acting as a positive regulator of differentiation in certain cell types in the brain.

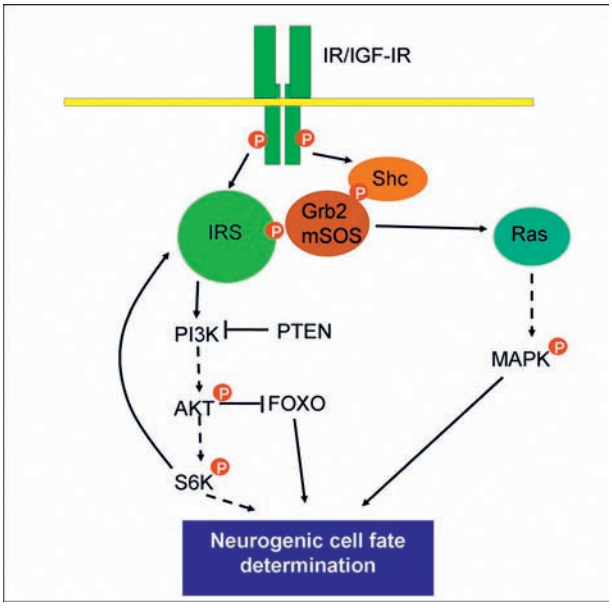

Figure 1. Potential pathways by which insulin/IGF signalling can regulate neurogenesis.

Insulin receptor signalling in *Drosophila*

Unlike vertebrates, *Drosophila* has a single RTK of the insulin receptor family (DInr). Expression of the DInr is ubiquitous during early stages of embryogenesis, but becomes enriched in the developing nervous system [32, 33]. The DInr can be activated by one of seven *Drosophila* insulin-like peptides (DILPS). Three of the DILPS are produced by seven neurosecretory cells within the brain. Flies in which these neurosecretory cells have been ablated are phenotypically similar to *dInr* mutants and have some features that are analogous to diabetes [34]. The DInr is required for growth during development and to attain full adult size [35]. Hypomorphic *dInr* mutants are developmentally delayed and have reduced size due to decreased cell number and cell size [36], suggesting that the role of the DInr during development is analogous to the IGF-IR. *dInr–/–* animals have defects in the development of embryonic central and peripheral nervous systems [32]. Unfortunately, this phenotype has not been studied in detail and so it is not clear whether embryonic neurons in *dInr* mutants are lost due to an inhibition of neurogenesis, proliferation, or indirectly though neuroblast apoptosis. In the developing eye, photoreceptor neurons do not absolutely require the DInr for neurogenesis; however, in the absence of the DInr, neuronal differentiation is significantly delayed [37]. Unlike activation of Ras/MAPK signalling, which is able to induce ectopic neurogenesis in the eye field, activation of DInr signalling modulates the timing of the differentiation programme. These findings suggest that the role of DInr signalling in neuronal differentiation is to act synergistically with other neurogenic pathways, such as EGF receptor (EGFR) signalling.

Does the DInr regulate the same intracellular signal transduction pathways as its mammalian counterparts? In *Drosophila* tissue culture cells, stimulation with mammalian insulin causes rapid phosphorylation of MAPK [38–40]; however, to date this has not been reported *in vivo*. Over-activation of MAPK signalling in the developing eye in *Drosophila* causes the formation of ectopic photoreceptor neurons [41, 42]. Over-expression of the DInr in the eye causes over-proliferation and, although the normal complement of photoreceptors are produced, there is a disruption in the patterning of the eye [36]. Interestingly, the patterning defect caused by over-expression of the DInr is similar to the planar cell polarity defects seen with mutations in EGFR signalling [43, 44], suggesting there may be cross-talk between these two pathways *in vivo*.

Chico, the *Drosophila* IRS, contains conserved putative binding sites for Drk, the homologue of the adaptor protein Grb2 [45]. Oldham et al. [46] generated transgenic flies containing a version of *chico* in which the putative Drk-binding site had been mutated, and found that this mutant was able to fully rescue the growth defects of *chico–/–* flies. In contrast, if the binding site for the regulatory subunit of PI3K (p60) in Chico was mutated, there was a complete loss of function. Why then is the Drk-binding site in the *Drosophila* IRS conserved? It is possible that a low level of MAPK activation may contribute to the ability of the DInr to control proliferation, although this is unlikely since loss of *pten* was able to completely rescue the growth defects caused by loss of the *dInr* [46]. Alternatively, the DInr may only activate MAPK in certain developmental contexts, such as embryonic development. Interestingly, loss of one copy of the *dInr* gene was able to dominantly suppress the embryonic lethality caused by over-expression of Ras^{V12} [47].

Work in the last few years has shown that, as in vertebrates, activation of the *Drosophila* PI3K is dependent on DInr signalling [28]. Signalling downstream of PI3K via AKT (PKB), the tuberous sclerosis complex (TSC) and TOR kinase is also highly conserved in *Drosophila*. As in mammals, the DInr pathway regulates the growth of flies via S6K and eIF4E. Moreover, the timing of photoreceptor neurogenesis in the developing eye is controlled by the DInr through a PI3K-TOR-dependent mechanism [37]. How might DInr signalling control neuronal differentiation through PI3K-AKT-TOR signalling? One of the targets of AKT is the forkhead transcription factor FOXO. FOXO regulates the transcription of a diverse set of genes that are involved in processes such as control of cell proliferation and apoptosis [48]. In certain developmental contexts, FOXO may be able to regulate the transcription of neurogenic genes, thereby mediating a neurogenic response to DInr stimulation. Alternatively, PI3K/TOR signalling may inter-connect with the Ras/MAPK pathway. Recent studies in mammalian tissue culture cells and in *Drosophila* have demonstrated the existence of a positive feedback loop by which S6K is able to regulate IRS levels and phosphorylation [49]. This feedback loop gives PI3K-AKT-TOR signalling the potential to control MAPK activation (and potentially neurogenesis) by modulating the activity of the IRS.

Conclusions and future directions

Can we assimilate the studies from vertebrates and flies to gain a greater understanding of the role of insulin/IGF signalling in neurogenesis? In both systems, *Ir*/*Igf1r* null animals show defects in CNS development. Further studies are needed, however, to characterise these defects in detail. Such studies should help to correlate the known expression patterns of the IR and IGF-IR with the affected neuronal/glial cell types. The mechanism of action by which insulin/IGF signalling controls differentiation is most easily addressed in cell culture systems. Vertebrate cell culture studies suggest that insulin/IGF-stimulated differentiation may occur through activation of the Ras/

MAPK pathway. Analogous studies have not been performed in *Drosophila* cells although the increasing availability of *Drosophila* neuronal cell lines in combination with RNAi technology provides an excellent opportunity to identify novel neural targets of the DInr. Vertebrate whole-animal models also show that insulin activates the Ras/MAPK pathway. *In vivo* studies in *Drosophila* have yet to demonstrate that the DInr can activate the Ras/MAPK pathway; however, our recent data suggest that in the *Drosophila* eye, the DInr pathway can regulate Ras/MAPK signalling through a transcriptional mechanism that requires TOR [unpublished results]. In conclusion, there is good evidence from both vertebrates and flies to suggest that insulin/IGF signalling has a conserved role in both proliferation and neuronal differentiation. The choice between proliferation and neurogenesis depends on the particular cell type or developmental context.

The contribution of insulin/IGF signalling to neurogenesis may be context and/or cell type specific; however, the importance of fine spatiotemporal control of neuronal differentiation means that understanding the role of this pathway is of major importance. Small alterations in the wiring of the brain can have profound consequences on function, and there are abundant data to suggest that the cues for axonal guidance alter over developmental time. In addition, the competence of neural progenitors to produce neurons of different fates is altered over time during development [reviewed in ref. 50]. To generate a structure of such intricacy as the brain, growth and differentiation must be coordinated, and the insulin/IGF signalling pathway appears to have just such a function. The challenge for the future is to understand molecularly how proliferation and differentiation are coordinated by a single pathway.

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