Developmental transcription factors in age-related CNS disease: A phoenix rising from the ashes?

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Developmental transcription factors in age-related CNS disease: a phoenix rising from the ashes?

Few would doubt that understanding the developmental landscape from which a mature neuron is derived is essential to understand its biology. The temporal and spatial position of a cell from the very earliest stages of development predicts the unique combinations of growth factors it will subsequently be exposed to. This combination of factors determines the transcriptional platform set within the cell by its specific combination of transcription factors, who direct the show. This, in turn, determines what cell type it will differentiate into, and what connections it will make. How this developmental platform translates to maintenance of a differentiated neuron in an adult brain is less clear.

Most developmental factors control aspects of biology that are not required, or even wanted, during or after the differentiation process: initiators of DNA synthesis or proliferation are clearly of no use to a differentiated neuron, and can certainly evoke damaging effects.

So, why is it commonplace in the adult brain to see the upregulation of developmental factors during stress, trauma, and disease? Two potential explanations dominate the debate. Firstly, that there is a cellular attempt to rejuvenate (the “phoenix from the ashes”) by using the only machinery it “knows” how, or secondly, that, indeed, these transcription factors are not simply ‘developmental’, and can drive distinct platforms of transcription in different circumstances (or “horse for many courses”). These two hypotheses are, of course, not necessarily mutually exclusive.

Regardless of the ‘motive’ many developmental transcription factor-encoding genes are recruited in difficult situations in later life. One such beast is Pax6, the subject of some of our laboratory’s research (Blake et al., 2008; Needhamsen et al., 2014; Thomas et al., 2016).

The developmental transcription factor Pax6: The Pax6 gene belongs to the highly functionally and structurally conserved Pax gene family (Pax1–9) of tissue-specific transcription factors. The Pax family are instrumental in development and have a critical role in brain regionalisation and specification of subtypes of neurons within brain regions. Pax6 is one of the earliest gene products expressed in the developing embryo. Initially, Pax6 is expressed in the neural plate, and after closure of the neural tube it is expressed in the lower ventral region except in the most ventral cells of the floor plate, acting to ventrally polarize the neural tube.

Pax6 is a key neurogenic factor and a well-accepted neurogenic determinant. Indeed, Pax6 is frequently used as a marker of neural precursor status. Recent studies have demonstrated that overexpression of both Pax6 and another transcription factor, Sox2, is sufficient to transdifferentiate fibroblast cells into induced neuronal progenitors (Maukensch et al., 2012), in line with it having been used the well-characterized rat 6-hydroxydopamine striatal lesion model, the number of Pax6-positive cells is significantly increased in the subventricular zone of adult wild-type mice maintaining mitotic and neuronal differentiation capabilities but undergo precocious differentiation resulting in cell fate switching and failure to generate specific dopaminergic neuronal sub-classes (Kohwi et al., 2005).

More research in this field is certainly required, as there are still many anomalies to overcome, as Pax6 suppression has also been shown to be beneficial in the generation of a dopaminergic phenotype from precursors (Denham et al., 2012).

In the adult brain: In the adult brain, Pax6 again rears its head, but “only” in very specific locations. Very specific indeed, and in keeping with its developmental role – in areas of continued proliferation and neurogenesis. Expression is maintained in adult neural progenitor cells of the subventricular zone of the olfactory bulb and the subgranular zone of the dentate gyrus (Stoykova and Gruss, 1994).

The “only” in the earlier sentence is in quotation marks as it is neither the whole truth nor is it nothing but the truth. When people have looked more exhaustively and in greater detail, isolated populations of Pax6-positive cells have been found. In the normal adult brain Pax6 expression has also been demonstrated in areas that do not undergo overt neurogenesis: the substantia nigra pars reticulata, retina, preoptic area, zona incerta, ventral tegmental area, and mesencephalic periaqueductal gray (Stoykova and Gruss, 1994; Vitalis et al., 2000). Pax6 is also expressed within certain post-mitotic cells of the adult substantia nigra in the human and rodent midbrain (Thomas et al., 2016). We have shown that, within the normal aging human adult midbrain substantia nigra, a very small number of cells express Pax6 protein. In the rat, many of these substantia nigra Pax6-positive cells co-label with tyrosine hydroxylase – the rate-limiting enzyme in the synthesis of dopamine and is expressed in catecholaminergic neurons (White and Thomas, 2012); and thus in this area of the adult brain, Pax6 is likely expressed in differentiated dopaminergic neurons. However not all Pax6-positive cells in the substantia nigra also expressed tyrosine hydroxylase, and we did not observe any co-labeling with astrocyte markers (glial fibrillary acidic protein), so this avenue of exploration certainly requires further work to generate a comprehensive identity of these cells.

Disease and damage: In keeping with its role in developmental neurogenesis, injury models have suggested that in these adult regions Pax6 expression is associated with the generation of new neurons in the areas of damage. For example a transient forebrain ischemic brain injury induces Pax6 up-regulation in subgranular zonal cells (Nakatomi et al., 2002); treatment of a rat Parkinson’s disease model with a dopamine receptor agonist increases proliferation and cell survival in newly generated neurons (Winner et al., 2009) with concurrent increased expression of Pax6 in these cells; a spinal cord injury results in transient up-regulation of Pax6 in the ependymal layer (Yamamoto et al., 2001); and the number of Pax6-positive cells is markedly increased in the subventricular zone following striatal quinolinic acid-induced striatal cell death in rats (Jones and Connor, 2011).

We have also recently shown that, in an animal model of Parkinson’s disease, the number of Pax6-positive cells within the midbrain substantia nigra increases significantly (Thomas et al., 2016). We used the well-characterized rat 6-hydroxydopamine striatal lesion model and assessed the number of Pax6-positive cells in the substantia nigra at time points corresponding to the phase of active cell loss (7 days post lesion) and after cell loss has plateaued (28 days post lesion).

In this model, the number of Pax6-positive cells is significantly
We obtained post-mortem brain tissue of people who died with Parkinson’s disease and have demonstrated that, in comparison to age and sex matched controls, the small number of cells expressing PAX6 in the human substantia nigra was significantly reduced.

So, what is it doing there? It seems that at least one important role that is directed by PAX6 in these adult brain cells is to ensure cell survival. Olfactory bulb neurons of 3 month old mice co-express PAX6 along with markers of terminal dopaminergic differentiation, including tyrosine hydroxylase and the dopamine transporter (Ninkovic et al., 2010). The loss of PAX6 expression by Cre-Lox recombination results in these neurons undergoing apoptosis, showing that PAX6 is required for the survival of some of these neurons (Ninkovic et al., 2010). Some of the genes targeted by PAX6 in this process are known; for instance PAX6 mediates survival of olfactory bulb neurons via crystallin αA and its loss reduces crystallin αA expression and induces apoptosis via caspase-3 activation (Ninkovic et al., 2010).

We also have recently shown that PAX6 effectively promotes survival in a cell culture model of PD – human neuroblastoma-derived SH-SY5Y cells differentiated down a dopaminergic lineage using retinoic acid and then exposed to dopaminergic-neuron-selective neurotoxins (rotenone and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). We induced expression of PAX6 after differentiation in this cell culture model and then applied neurotoxic insults and measured survival, apoptosis and mitochondrial depolarization. PAX6 induction markedly improved how the cells fared in all these parameters, indicating that PAX6 over-expression following differentiation increases the survival of SH-SY5Y cells exposed to dopaminergic-neuron-selective neurotoxins by increasing their resistance to both programmed cell death and the effects of oxidative stress on mitochondrial health and function. It is worth noting that we also observed a significant upregulation of another developmental transcription factor gene known to play a role in the differentiation and survival of midbrain dopaminergic neurons, neurogenin 2, following PAX6 induction (Thomas et al., 2016).

It is intriguing to note that in Parkinson’s disease, the two substantia nigra areas, the pars compacta and the pars reticulata do not share equal vulnerability to developing pathology; the substantia nigra pars compacta experiences the highest levels of degeneration, with the substantia nigra pars reticulata degenerating later as the disease progresses. As we have shown, PAX6 expression occurs in the substantia nigra pars reticulata, which could imply a role for PAX6 in the protection of these dopamine-producing cells. Importantly then, PAX6 may have a third function in promoting cell maintenance and/or survival of adult neurons (Blake et al., 2008).

Conclusion: The phoenix of Greek mythology is a long-lived bird that is cyclically regenerated or reborn, obtaining new life by arising from the ashes of its predecessor. We do not know the precise function of our ‘phoenix’ developmental transcription factors in the neurobiology of aging brains of humans yet, but it is not entirely unlikely that one day we will use them to enable rebirth within the ashes of the degenerating central nervous system.

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We dedicate this work to the memory of the wonderful Oliver Sacks R.I.P. May his legacy continue to awaken the minds of readers for many years to come.

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