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

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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 (Maukisch et al., 2012), in line with it having been demonstrated that Pax6 alone induces neuronal specification of postnatal forebrain astrocytes (Heins et al., 2002).

As well as specifying neural progenitor cells, Pax6 also acts in a second phase of fate decision-making during development, namely during the specification of a subpopulation of neurons. Within the developing brain, Pax6 expression is confined to the forebrain, optic vesicles and the ventral midbrain (Stoykova and Gruss, 1994). Its expression correlates with the appearance of dopaminergic neurons of the ventral thalamus (preoptic area, zona incerta and periventricular and arcuate nuclei), the mesencephalic tegmentum (region of the differentiating substantia nigra) as well as in neurons of the dorsolateral part of the reticular substantia nigra (Stoykova and Gruss, 1994; Vitalis et al., 2000).

Over-expression of Pax6 in a variety of cells and circumstanc-
elevated during the phase of active cell loss, and this increase in Pax6-positive cells is maintained after cell loss has completed. This potentially indicates that the cells that upregulate Pax6 do not die throughout this process, and as we have shown that many of these Pax6-positive cells are differentiated dopaminergic neurons (tyrosine hydroxylase-positive), then we believe this is indeed an interesting finding warranting further investigation.

So what about the human disease ‘model’? 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. But Pax6 expression is reduced in the substantia nigra pars reticulata, which could imply a role for Pax6 in the protection of these dopamine-producing cells. Importantly, 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, neuregulin 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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