Neural stem cell dynamics: the development of brain tumours

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Abstract

Determining the premalignant lesions that develop into malignant tumours remains a daunting task. Brain tumours are frequently characterised by a block in differentiation, implying that normal developmental pathways become hijacked during tumourigenesis. However, the heterogeneity of stem cells and their progenitors in the brain suggests there are many potential routes to tumour initiation. Studies in *Drosophila melanogaster* have enhanced our understanding of the tumourigenic potential of distinct cell types in the brain. Here we review recent studies that have improved our knowledge of neural stem cell behaviour during development and in brain tumour models.

Introduction

Neural stem cell (NSC) division is required to generate neurons and glia at the right time and place, but aberrant NSC proliferation can lead to tumourigenesis. Therefore, a balance between NSC proliferation and differentiation must be maintained during development and in adulthood. Tumours affecting the central nervous system (CNS) are among the most poorly understood and difficult to treat. The recent identification of tumour-specific genetic markers, in combination with histopathological analysis, has allowed for the classification of CNS tumours [1]. While these developments have provided important diagnostic tools, they have not necessarily led directly to therapies and many brain tumours remain resistant to available treatments. This is due, in part, to the enormous diversity of CNS tumours, with both inter- and intra-tumour heterogeneity, as well as a lack of knowledge regarding the molecular basis of tumour malignancy. It is challenging to determine the pre-malignant lesions that progress to malignant tumours in humans, but much can be learned from studying simpler organisms that employ conserved mechanisms to regulate NSC behaviour.

Here we summarise recent findings on brain tumour initiation and malignancy in *Drosophila*, which has served as a valuable cancer model for many years [2]. The unparalleled genetic toolkit for *Drosophila* has enabled diverse aspects of tumourigenesis to be investigated.

1. The diversity of *Drosophila* neural stem cells

The relative simplicity of the *Drosophila* CNS provides an excellent model system in which to investigate the mechanisms that regulate neurogenesis [3]. *Drosophila* neurogenesis occurs in two distinct phases: (1) an embryonic phase, that generates the larval nervous system, and (2) a larval phase, that generates the adult nervous system [4]. The majority of *Drosophila* NSCs (called neuroblasts) arise from the neuroectoderm during embryogenesis and exhibit three main division modes: type 0, type I and type II (**Fig. 1A-B**) [5]. Type I neuroblasts are the most prevalent NSC in the embryonic and larval CNS. Asymmetric division of a type I neuroblast results in NSC self-renewal and the generation of a ganglion mother cell (GMC), a cell type that undergoes a terminal division to produce two neurons or glial cells. At the end of embryogenesis, a small number of type I lineages switch to a type 0 division mode, whereby their daughter cells (GMCs) do not divide, but differentiate directly to a neuronal fate [6-8].

Type II neuroblasts, of which there are eight in each brain lobe, exhibit the highest self-renewal capacity in the developing CNS and represent a rare NSC state. Asymmetric division of type II neuroblasts generates intermediate neural progenitors (INPs) [9-11], which retain many NSC properties, such as the ability to self-renew and produce GMCs that give rise to neurons or glia. Importantly, the embryonic origin of type II neuroblasts and their lineages has recently been described [12,13]. These studies identified the genes that promote type II neuroblast fate during embryogenesis [12], with roles for *buttonhead*, *Sp1* and the EGFR pathway, and determined the contribution these embryonic NSCs make to the adult central complex [13].

Yet another type of NSC generates the neurons and glia of the adult visual system (the optic lobe) and has a different origin. These NSCs arise from symmetrically dividing

neuroepithelial cells that are converted to asymmetrically dividing neuroblasts by a wave of proneural gene expression that traverses the larval optic lobe [14]. The majority of optic lobe NSCs divide in a type I manner, with the exception of a small population at the tips of the neuroepithelium that exhibit type 0 divisions [15].

While the development of the optic lobe during larval development has been well characterised, the behaviour of optic lobe NSCs during embryonic development has recently been revised [16]. The optic lobe neuroepithelium was thought to be dormant during embryogenesis, but this view has been overturned by the discovery of a small number of NSCs that are generated by the embryonic optic lobe neuroepithelium (embryonic optic neuroblasts, EONs) [16]. Although the contribution of EONs to visual system development and their tumourigenic capacity are yet to be determined, their identification highlights the dynamic behaviour of NSCs in the optic lobe during the early stages of development.

2. Neural lineages as the source of tumours

The asymmetric division of NSCs is a fundamental aspect of cell biology that underlies neurogenesis. The generation of two cells with different identities after cell division can arise from the unequal segregation of cell fate determinants at mitosis. In *Drosophila* NSCs, fate determinants have been observed to localise on the basal side of the cell to enable partitioning to their daughter cells (GMC or INP, depending on the lineage) (**Fig. 2A-A'**). In all NSCs, the adaptor protein Miranda (Mira) is a central player in the segregation of cell fate determinants and correct localisation of Mira establishes daughter cell identity [17-21]. Recent studies have revealed that the phosphorylation state of Mira is important for cortical localisation [22]. During interphase, Mira is non-phosphorylated and is uniformly associated with the plasma membrane, but aPKC-mediated phosphorylation at metaphase prevents the localisation of Mira at the apical pole [22,23]. Furthermore, the asymmetric localisation of Mira is maintained through interactions with its cognate mRNA [24]. Robust Mira localisation ensures that cell fate determinants are distributed to the GMC or INP when a NSC divides [18-21]. Disrupting Mira localisation at asymmetric NSC division, for example through the ectopic expression of activated aPKC, impairs daughter cell differentiation and results in ectopic NSCs [18,25,26]. It is also notable that the activation of aPKC has been shown to promote hyperplastic

growth of the neuroepithelium [27].

Two cell fate determinants that associate with Mira are the homeodomain transcription factor Prospero (Pros) and the translational repressor Brain tumour (Brat) [17-19]. Consistent with the loss of function phenotype of *mira*, mutations affecting either *pros* or *brat* result in an increase in NSCs [28,29]. However, *pros* tumours are found throughout the CNS [28] whereas *brat* tumours affect only the central brain [29]. This difference occurs because *pros* and *brat* mutations initiate tumourigenesis from different cells of origin (**Fig. 2B**). *brat* tumours arise from INPs of type II lineages that fail to differentiate [11,30]**,** whereas GMCs revert to NSC identity in the absence of *pros* [28].

Many of the tumour suppressors identified in *Drosophila* initiate tumourigenesis from neural progenitors. A number of these tumours originate specifically from the INPs of type II lineages. The adaptor protein Partner of Numb (Pon) is responsible for partitioning the Notch antagonist, Numb, to INPs at mitosis [31]. The loss of Numb prevents the establishment of INP fate and leads to large tumours consisting of type II neuroblasts, similar to the phenotype observed in *brat* mutants The FezF transcription factor Earmuff (Erm) regulates the maturation of INPs and mutations in *erm* result in the dedifferentiation of INPs to type II neuroblast fate [32-34]. Intriguingly, a recent study has suggested that the decision for INPs to differentiate or revert to NSC fate in certain *brat* mutants could be stochastic [35]. The majority of the proliferating cells in these tumours are not type II neuroblasts but are likely INPs that maintain the ability to self-renew and produce neurons and glia [35]. As such, INPs represent a weak point in type II lineages that is susceptible to distinct tumourigenic insults. Type I lineages, in contrast, are unaffected by mutations in *brat*, *numb* or *erm* [11,32]**.**

Mutations in the gene *lethal(3)malignant brain tumour* (*l(3)mbt*) result in the overproliferation of neuroepithelial cells in the optic lobe without affecting asymmetric cell division [36]. L(3)mbt is a chromatin insulator that binds to many Hippo pathway target genes to repress their expression and restrain proliferation [36]. Surprisingly, *l(3)mbt* brain tumours ectopically express many germline genes that are required for tumour malignancy, including homologues of human cancer/testis antigens [37].

Remarkably, even post-mitotic cells have been shown to revert to NSC fate and serve as the tumour cell of origin. Mutations in the gene encoding the BTB zinc finger transcription factor Longitudinals lacking (Lola), results in the dedifferentiation of optic lobe neurons to a NSC-like fate [38]. Similarly, mutations affecting Nerfin-1 result in ectopic NSCs originating from neurons of type I and type II lineages [39]. These experiments demonstrate that neurons are able to reinitiate a stem cell programme to become tumour cells of origin, as can GMCs and INPs, highlighting the diverse nature of tumour initiation within NSC lineages (**Fig. 2B**). Similar findings have been made in mouse tumour models, which have shown that malignant gliomas can also originate from differentiated neurons and suggest that tumour initiation likely occurs via dedifferentiation [40].

3. Temporal patterning and tumour malignancy

NSCs divide continuously throughout larval development to generate neurons and glial cells but the identity of the neuronal subtypes they generate changes over time. The birth order and identity of their progeny is directed by the sequential expression of a series of 'temporal transcription factors' [41-43]. The temporal patterning of NSCs also ensures their timely cell cycle exit at the end of larval development [44,45]. In contrast, ectopic NSCs that result from the loss of many tumour suppressor genes do not exit the cell cycle but rather have an extended capacity to self-renew [25]. The unlimited proliferative potential of tumour NSCs was demonstrated through serial transplantation experiments, in which pieces of larval brain tumour were allografted into the abdomen of adult host flies and could be maintained in this manner for years [25]. It may not be surprising that mutations affecting asymmetric cell division lead to an increase in NSCs, but the ability of these ectopic NSCs to divide indefinitely showed that they were truly tumourigenic and not simply hyperplastic overgrowths. However, the mechanisms through which tumour NSCs maintain an unrestricted proliferative potential, far beyond that of their developmental counterparts, was not clear. Recent evidence has implicated the temporal identity of the tumour cell of origin as a prerequisite for tumour malignancy [46].

Two broad temporal windows play an important role in tumour malignancy: (1) an early window, defined by the expression of Chronologically inappropriate

morphogenesis (Chinmo), IGF-II mRNA-binding protein (Imp) and Lin-28, and (2) a late window, characterised by the expression of Syncrip (Syp), Broad (Br) and Ecdysone-induced protein 93F (E93) (**Fig. 3A**) [45-48]. The loss of *pros* from NSC lineages during either temporal window results in large NSC tumours. However, tumours induced early in larval development are maintained as large tumours in the adult nervous system (**Fig. 3B**), whereas those generated in the late temporal window mostly differentiate (**Fig. 3C**) [46]. Intriguingly, the authors found that the majority of tumour NSCs progressed from early to late identity during larval stages, at the same as surrounding wild type tissue, but a small population of tumour NSCs retained early identity. These early-identity tumour cells were responsible for the malignancy of *pros* tumours, driving tumour growth and persistence in the adult [46,49].

How does early NSC temporal identity promote tumour growth? An important difference between the early and late temporal windows during development is the competence of NSCs to respond to the steroid hormone ecdysone [47]. Ecdysone levels increase toward the end of larval stages and signal NSC cell cycle exit [44,50]. The expression of the B1 isoform of the ecdysone receptor (EcR) in NSCs is regulated by the orphan nuclear receptor Seven-up (Svp) (orthologous to mammalian COUP-TF transcription factors) coincident with the early-to-late transition, which is also regulated by Svp [45,47,51]. Intriguingly, ecdysone signalling has also been shown to regulate the early-to-late transition [47], although these results could not be replicated in another study [52]. The subpopulation of *pros* tumour NSCs that are stalled in the early temporal window do not respond to the endogenous signals, such as ecdysone, that stimulate NSCs to exit the cell cycle [46,49]. As a result, these tumour cells continue to divide in the adult and drive tumour growth.

The role of early temporal identity in enhancing tumour growth is not unique to *pros* tumours. In *brat* tumours, a subpopulation of tumour NSCs that express the early temporal genes Chinmo and Imp also drives tumour malignancy, and the repression of these genes restricts tumour growth [46,53]. One study identified a long non-coding RNA (lncRNA), *cherub*, that promotes tumour malignancy by preventing the temporal progression of tumour NSCs [53]. It is also notable that NSC hyperplasia induced by the ectopic activation of Notch signalling in type II INPs declines with the age of the cell of origin [54].

The identification of specific subsets of neural cells that promote tumour growth in the *Drosophila* CNS provides support for the cancer stem cell (CSC) hypothesis. CSCs are proposed to drive tumour malignancy and regrowth following treatment due to their resistance to traditional therapies [55]. The recent studies reviewed here take advantage of the simple *Drosophila* nervous system to identify conserved factors that promote the formation and maintenance of CSCs *in vivo*.

Concluding remarks

Studies in *Drosophila* have revealed that many distinct tumour types can arise from neural lineages. Furthermore, it is clear that tumours with common pathologies may develop from different cells of origin. Neural lineages are also the source of tumours affecting the mammalian CNS, in particular medulloblastoma and glioblastoma, which are two of the most aggressive brain tumours (reviewed in [56]). Evidence suggests that mammalian tumours may arise, not from NSCs directly, but from more restricted cells in the lineage [57-60] indicating that dedifferentiation is a conserved mechanism of tumour initiation.

It remains unknown how the temporal identity of the cell of origin influences tumour malignancy in humans, and this will be an important aspect for future study. The recent adoption of single cell sequencing methods has made it possible to determine the transcriptomes of large numbers of cells and to profile the diverse cell types found in the developing and adult mammalian brain [61-63]. Promisingly, this approach has demonstrated that subtypes of childhood cerebellar cancers have very similar transcriptional profiles to distinct foetal progenitors found only early in development [64]. This suggests that the developmental window of tumour induction could determine the composition of mature tumours, but detailed lineage analysis *in vivo* will be required to assess if this is indeed the case for mammalian brain tumours.

Conflict of interest statement

The authors declare no conflict of interest.

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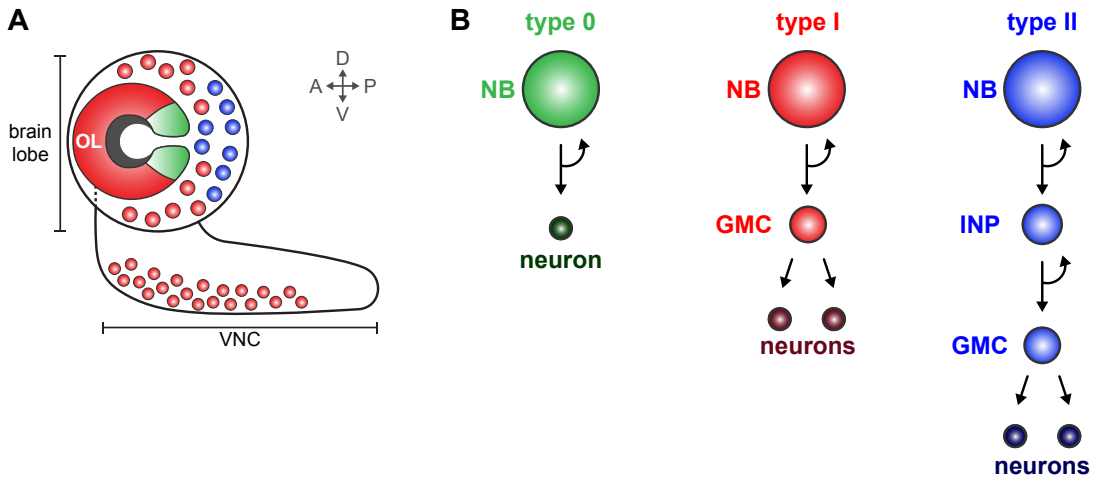

Figure 1: Neural stem cells in the developing *Drosophila* central nervous system

(**A**) NSCs in the developing *Drosophila* CNS (also called neuroblasts) are organised in stereotypical locations across the brain lobes and ventral nerve cord (VNC). The schematic shows a brain from mid/late larval stages. The majority of neuroblasts are type I (red). The optic lobe (OL) contains mostly type I neuroblasts but also a subset of type 0 neuroblasts (green). Type II neuroblasts are found in the posterior region of the brain lobe (blue). A, anterior; P, posterior; D, dorsal; V, ventral.

(**B**) Division mode of *Drosophila* neuroblasts (NB). GMC: ganglion mother cell, INP: intermediate neural progenitor.

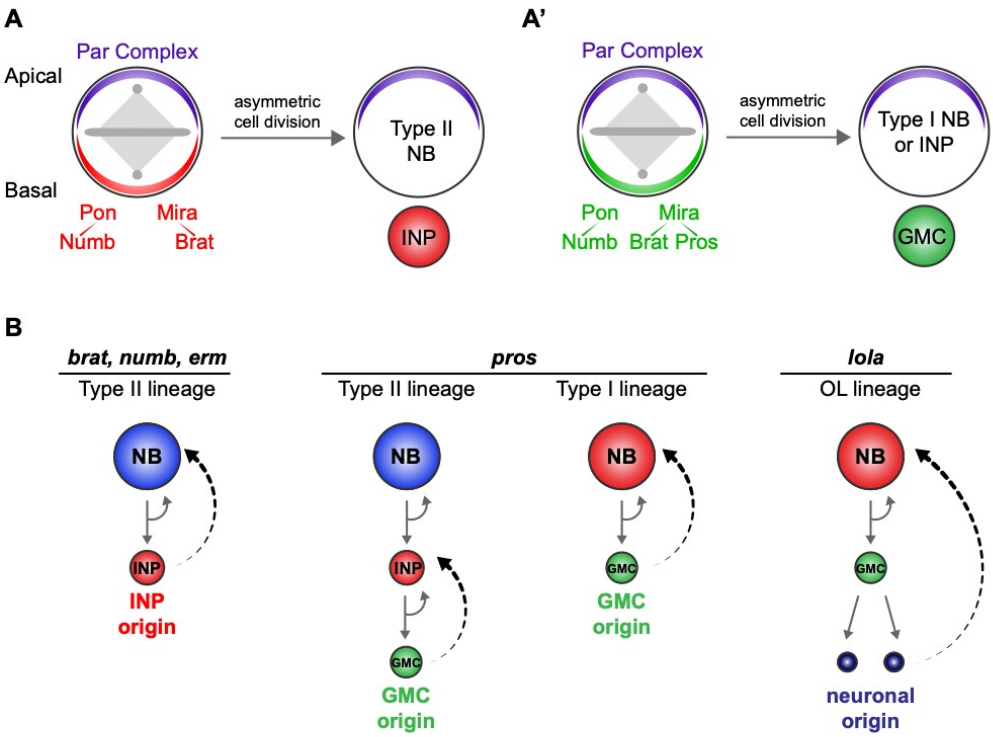

Figure 2: Neural stem cell lineages as tumour cells of origin

(**A-A'**) The unequal distribution of fate determinants establishes differences in cell identity in the nervous system. In neuroblasts, members of the Par complex (aPKC/Bazooka/Par6) localise apically whereas cell fate determinants are anchored basally through interactions with adaptor proteins. (**A**) In type II neuroblasts, the adaptor protein Mira localises Brat to the basal cortex and Numb is recruited by the adaptor protein Pon. (**A'**) In type I neuroblasts and INPs, Brat and Numb are localised asymmetrically but do not regulate GMC fate. In contrast, correct distribution of Pros, regulated by Mira, is required for establishing GMC fate.

(**B**) Mutations affecting different cell fate determinants initiate tumours from distinct cells of origin.

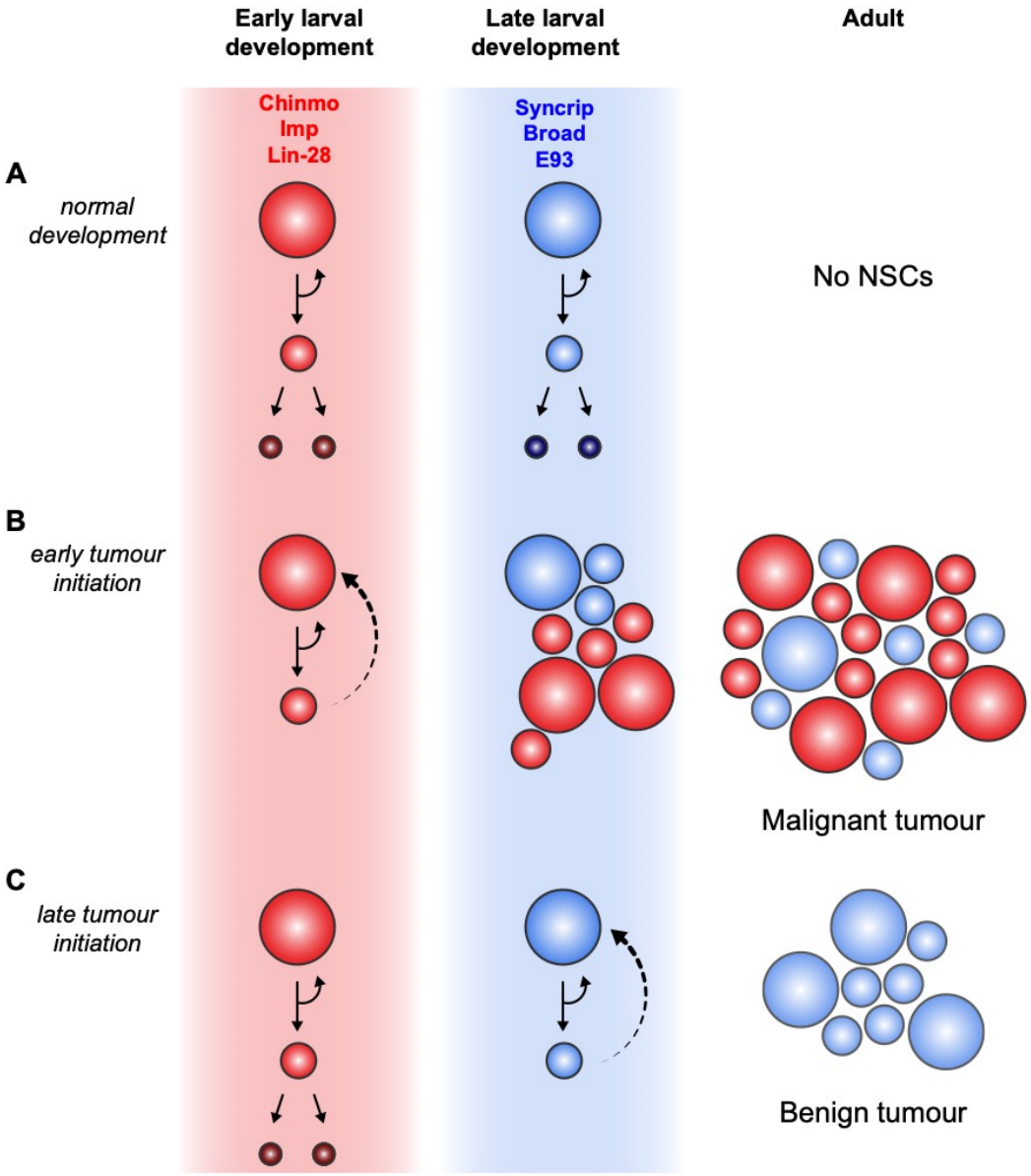

Figure 3: The temporal identity of the cell of origin determines tumour malignancy

(**A**) Temporal progression of NSCs and their lineages during development.

(**B**) Induction of NSC tumours in the early temporal window results in malignant tumours that are maintained in the adult.

(**C**) In contrast, a large number of NSCs in tumours induced during the late temporal window undergo differentiation before reaching the adult. These tumours show restricted growth and are characterised as benign.

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•• The relationship between the tumour cell of origin and the composition of malignant tumours is not clear for human cancers. This paper compared single cell RNA sequencing of the mouse cerebellum, from embryonic or postnatal stages, with the transcriptomes of human childhood cerebellar tumours. The findings suggest that the cell of origin determines the cell types present in mature tumour and suggests that temporal progression is defective in tumour cells.