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Glioblastoma treatment using perphenazine to block the subventricular zone's tumor trophic functions

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Abstract We present here a potential new treatment adjunct for glioblastoma. Building on murine studies, a series of papers appeared recently showing that therapeutic irradiation of the ipsilateral subventricular zone (SVZ) retards growth of more peripherally growing cortical glioblastomas in humans, suggesting a tumor trophic function for the SVZ. Further studies showed that SVZ cells migrate out towards a peripheral glioblastoma. Dopamine signaling through D3 subtype receptor indirectly drives this centrifugal migration in humans. Since psychiatry has several drugs with good D3 blocking attributes, such as fluphenazine, or perphenazine, we suggest that adding one of these D3 blocking drugs to current standard treatment of resection followed by temozolomide and irradiation might prolong survival by depriving glioblastoma of the trophic functions

previously subserved by dopaminergic signaling on SVZ cells.

Keywords Dopamine receptor · Glioblastoma · Growth factors · Migration · Perphenazine · Subventricular zone · Temozolomide · Trophic factors

Introduction

To improve the currently poor prognosis of glioblastoma we have been pursuing principally new approaches using already-marketed drugs to block identified glioblastoma growth-promoting pathways [1–3]. In further pursuit of this goal, we add the current paper.

The subventricular zone (SVZ) refers to a several cell layer thick periventricular area, particularly within the lateral walls, delimited on one side with the single cell layer of ependymal cells that line and are in contact with cerebrospinal fluid in the lateral ventricles, and on the other side by cortical structures [4, 5]. SVZ cells proliferate, albeit slowly, throughout life, providing the two brain sources of new neurons and glia [4, 6], the only other source being dentate gyrus hippocampal subgranular zone [7].

Although not firmly established as correct, it has been postulated that the SVZ has a special role in human glioblastoma growth [8, 9]. We review past research data leading to this postulate and show an immediately applicable treatment consequence that would arise if the special role of the SVZ in glioblastoma growth would be confirmed in due course.

The ependymal cells lining the lateral ventricles, normally quiescent non-dividing cells, can be driven to asymmetric division by growth factors such that one daughter cell migrates into the SVZ, the other daughter

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resting within or near the ependyma [10]. In normal adult mammals, neuronal precursors can be seen to divide mainly in the SVZ and the dentate gyrus hippocampal subgranular zone [5]. SVZ cell proliferation is easily documented in mice after dopaminergic agonists [11].

Previous studies indicate that dopamine signaling through the D3 receptor promotes SVZ cell mitosis and induces SVZ cell centrifugal migration under normal conditions. We believe this D3-driven migration towards peripheral glioblastomas serves a trophic function for this tumor. Depriving a glioblastoma of SVZ derived cells' trophic function might not by itself defeat a glioblastoma but not stopping this function might prevent our defeating it by other means.

Since we already have several psychiatric drugs on the market with D3 antagonism properties, for example fluphenazine, or perphenazine, we suggest a trial in glioblastoma models where SVZ contribution to growth has been established. Fluphenazine or perphenazine are cheap generic drugs in wide use throughout the world since the 1950s, usually adequately tolerated by non-psychiatric as well as psychiatric patients. Given the documentation we review here that the SVZ contributes to glioblastoma growth and that this contribution is at least partially dependent on D3 receptor action, we may indeed have a ready-made treatment adjunct in perphenazine.

SVZ importance in glioblastoma

Evidence from murine studies

Multiple study approaches in murine glioblastoma models have demonstrated a particular vulnerability to oncogenic stimuli in cells residing in the SVZ and the hippocampal dentate gyrus [12]. When human glioblastomas are transplanted into the cortex of nude mice, murine SVZ cells become integrated into the resultant tumor [13]. Both cell lines derived from an SVZ-close and an SVZ-far human glioblastoma grew better when injected in nude mouse SVZ compared to when they are injected into host cortex [14]. Clear centripetal tropism can be demonstrated (inwards towards and to the SVZ) when human glioblastoma cells are injected near but peripheral to the SVZ of athymic mice [15]. Rat SVZ cells proliferate *in vivo* in response to the D2 < D3 agonist pramipexole, progeny later appearing as well-differentiated olfactory bulb neurons [16]. Ample D3 receptor mRNA is present on mature murine SVZ cells, but importantly only on selected cells within the SVZ [17].

Human clinical evidence

Chen et al. [18], acting on the SVZ importance hypothesis of glioblastoma growth, determined that in 116 patients

Fig. 1 Occurrence frequency map taken from initial MRIs of 380 glioblastomas. Direct proportionality, increasing incidence location to proximity to the SVZ, can clearly be seen. SVZ subventricular zone, CC corpus callosum, LV lateral ventricle, CB cerebellum

who were able to have total resection of their glioblastoma, marginally longer overall survival (OS) was associated with the ipsilateral SVZ receiving ≥ 40 Gy irradiation compared to similar gross total resection patients receiving less than 40 Gy to their SVZ [18]. In 40 post-resection cases, progression-free survival (PFS) was 11 months and OS 17 months, but those receiving higher doses to the ipsilateral SVZ survived longer [19], confirming a previous 2010 study that retrospectively looked at calculated doses received by the SVZ showing 15 versus 8 month OS when higher ipsilateral radiation doses are given [20]. Lee et al. [21] found indications of similar longer OS after higher irradiation doses received by the ipsilateral SVZ, though their results were of borderline statistical significance.

In an MRI study of 507 glioblastomas, where images were registered to a standard 1.0-mm isotropic brain atlas, Ellingson et al. [22] found that 92 % of cerebral glioblastomas had signal abnormalities extending to the SVZ, concluding, “Most glioblastomas grow into the periventricular white matter regions adjacent to the subventricular zone”, particularly so in the posterior SVZ [23]. However, “grow into” might not be correct in that their data does not allow any firm conclusion about which way the communication was established, SVZ glioblastoma cells migrating to the peripheral more prominent tumor mass or from that mass to the SVZ—a modification of their original wording with which lead author on that paper and co-author here, Ben Ellingson, agrees.

In Fig. 1 we show a statistical map of occurrence location drawn from 380 cases of primary glioblastoma. This clearly shows increasing incidence with progressive proximity to the SVZ. In Fig. 2, representative glioblastoma MRIs

Fig. 2 Representative contrast-enhanced MRIs from 24 patients where contact points to the SVZ can be easily observed, indicated by *red arrows*

indicate points of contact to the SVZ by red arrows, taken from 24 untreated cases.

A morphometric MRI study of 39 newly-diagnosed glioblastomas showed a direct relationship between proximity to the SVZ and shorter OS [7]. Patients with glioblastomas having easily demonstrable contact with the SVZ had shorter OS than those not in such contact (358 vs 644 days) [24].

An instructive and potentially clinically meaningful perioperative MRI study of 53 cases of primary glioblastoma divided these into four groups by the tumor's relationship to the SVZ. Group 1, where the primary tumor mass contacted

the SVZ and cortex; group 2, where the primary mass contacted the SVZ but not the cortex; group 3, where the primary mass did not contact the SVZ but was entirely within the cortex; and group 4 where the main tumor mass neither contacted the SVZ nor the cortex [25]. In group 1, 56 % presented with multifocal disease while group 4 never had multifocal disease. In group 1, 100 % had recurrence non-contiguous with the original mass while 100 % of group 4 recurrences bordered the original mass [25], a finding that suggests more of an originating function than trophic function for the SVZ. The question remains open whether the SVZ has a trophic function or originating function.

SVZ and D3 receptors

Dopamine acting through the D3 receptor stimulates the SVZ to proliferate *in vitro* [26]. D3 receptors are however not active in promoting SVZ cell centrifugal migration or division in all species. While confirming such D3 activity in rats, Baker et al. [27] showed D3 agonism in mice results in neither SVZ cell migration nor mitosis. Why others did find murine SVZ cell migration and mitosis in response to dopaminergic stimuli [11, 28] is unclear.

Two D2–D3 blocking drugs in common use in humans to treat psychosis, haloperidol and sulpiride, blocked dopaminergic agonist-induced *in vitro* proliferation of SVZ cells taken from normal adult rats [29]. *In vitro* haloperidol or sulpiride cytotoxicity to these SVZ cells was not seen [29].

In vitro SVZ cell proliferation stimulated by dopaminergic agonists appears to be mediated by an EGF obligate step [30]. Further evidence implicates proteolytic cleavage release of autocrine or paracrine outer cell membrane-tethered EGF as the EGF source in dopaminergic SVZ stimulation [31].

Pramipexole is a non-ergot dopaminergic agonist approved for use in stopping unwanted lactation and Parkinson's disease. It has eightfold greater agonist affinity for D3 compared to D2 receptor. *In vitro* exposure of murine SVZ cells to pramipexole stimulates mitosis that was inhibited by the D2–D3 (D2 < D3) inhibitor sulpiride [28].

The drug: treatment consequences

Perphenazine is a generic, well-characterized medicine used primarily in treatment of psychosis [32], introduced into clinical psychiatric practice in the 1950s and in continuous use since then [33]. Perphenazine readily penetrates the blood brain barrier and usually is adequately tolerated in the non-psychiatric population [34]. Perphenazine also is a reasonably effective anti-emetic, anti-nausea agent [34] and a common depression treatment adjunct [35]. Strong D3 blocking and favorable ratio of D2–D3 blocking of perphenazine [36] would suggest its use to deprive human glioblastomas from whatever trophic functions the SVZ may be providing.

Caveats and discussion

Ongoing SVZ generation of neural and glial precursors is a feature of normal adult mammalian brain functioning. Although we reviewed above data indicating that irradiation of the ipsilateral SVZ retards the growth of a glioblastoma growing centimeters distal to the SVZ, implying a

tumor trophic function of SVZ cells, potentially conflicting data exist. Glass et al. [37] found a glioma growth-inhibiting effect of centrifugally migrating SVZ cells. While confirming SVZ cell migration/tropism toward a peripherally implanted glioma, the same group showed that the glioma growth-inhibiting effect was due specifically to neural-precursor SVZ cells [38].

Dopamine is currently recognized as signaling through five different receptors grouped into D1-like (D1 and D5) and D2-like (D2, D3, D4) [39]. Intracellular responses to dopamine signaling through D2-like receptors is amplified when these receptors dimerize [hetero or homo] compared to their equimolar monomeric forms [39, 40]. Such D2–D3 dimerization-related signaling potentiation could account for the discrepant data on D2 versus D3 importance in driving SVZ cell migration or mitosis. Again, perphenazine might be a good choice in either case given its balanced and strong inhibition at both D2 and D3 [36]. Vilner et al. [41] noted perphenazine was cytotoxic at high concentration to C6 glioma cells *in vitro*, ascribing this to agonism at sigma receptors. Many sigma agonists are already marketed for other indications and have been suggested to have anti-glioblastoma activity [42], and indeed in a follow-up study many sigma-1 agonists showed good cytotoxicity to glioblastoma cell lines both *in vitro* and in orthotopic xenotransplant work [43].

Complexity of primate SVZ architecture compared to that of rodent SVZ as reviewed by Betizeau et al. [44] at the University of Lyon show that primates have a richer array of SVZ resident precursor cells, many of which show bidirectional differentiation ability [44, 45]. Which SVZ subset are triggered to exit quiescence by dopaminergic signaling and which if any are not would be an important matter to determine both for development of neurotherapeutics generally and for a more detailed delineation of glioblastoma subtypes vis-à-vis their corresponding SVZ subpopulation. Differences between man and mouse exist in the structure, regulation, and directional tropism but importance of dopaminergic signaling in driving exit from quiescence remain evident for both [17, 44, 45].

Conclusion

The relationship between glioblastoma and the SVZ remains unclear. We have reviewed research evidence indicating that dopamine signaling to SVZ cells is a component of triggering their exit from quiescence and centrifugal migration. Research indicates both dopamine stimulated centrifugal migration of activated SVZ cells and centripetal migration of glioblastoma cells toward the SVZ. We conclude that either way the SVZ has potential to serve a trophic function to a more peripherally growing

glioblastoma. We reviewed evidence indicating a glioblastoma-generating function originating in the SVZ. Given the current impasse in glioblastoma treatment, we have been exploring medical treatments that, while not cytotoxic in themselves, will undermine a cancer's survival strategies that are engaged by exposure to our cytotoxic interventions [1–3]. To these studies, we now suggest adding perphenazine to undermine dopaminergic-driven SVZ cell contributions to glioblastoma growth.

Conflict of interest None of the authors has conflict of interest to declare.

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