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Neural stem cells as novel cancer therapeutic vehicles

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ABSTRACT

The startling resemblance of many of the behaviours of brain tumours to the intrinsic properties of the neural stem/progenitor cell has triggered a recent dual interest in arming stem cells to track and help eradicate tumours and in viewing stem cell biology as somehow integral to the emergence and/or propagation of the neoplasm itself. These aspects are reviewed and discussed here.

1. Intracranial tumours: primary and secondary

The origin of primary intracranial tumours is not yet clear. The striking similarity between the behaviour of normal neural stem cells (NSCs) and neoplastic cells of neuroectodermal origin,[1] at least in terms of migratory capacity, ability to insinuate themselves into normal tissue, self-renewal potential, and molecular signature, first gave rise to the notion that they may constitute 'two sides of the same coin', the latter having lost the growth and differentiation control mechanisms of the former. It appears, in fact, that the virulence of intracranial tumours is maintained by the presence within the tumours of a population of 'stem-like' cells.[2–6] It remains unclear whether the brain tumours and their 'cancer stem cells' (as they have been dubbed) emerge from the de-differentiation of mutated mature neural cells or emanate de novo from otherwise normal organogenic progenitors fated or predisposed (early in development, at a pre-tumourigenic phase) to become neoplastic. 'Circumstantial' experimental evidence supports both as potential aetiologies, while not clearly clinching either scenario.[7]

Gene expression microarray technology has now allowed for the classification and identification of subsets of tumours, based on the differential expression of sets of genes which may portend differences in disease progression and patient survival[8] as well as suggesting potential therapeutic targets based on the identification of oncogenic molecular path-

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ways.[9,10] For example, a 'favourable' genetic profile, such as loss of heterozygosity of chromosome 1p and/or 19q in oligodendrogliomas, and methylation status of the O-6-methylguanine-DNA methyltransferase (MGMT) promoter in gliomas, identified patients with better prognosis and a more favourable response to chemotherapy.[11–13] Conversely, the expression of stem-cell like genes in different human tumours appeared to correlate with a poorer prognosis.[14]

Similarly, a population of putative brain tumour stem cells (BTSC) expressing the cell surface antigen prominin-1 (CD133) was isolated and characterised from surgical resection specimens.[4,5] The most important property of this subpopulation was its ability to generate secondary tumours when implanted into naïve animals, i.e. one suggestion of self-renewal capacity. That CD133 is expressed by BTSC isolated from both human gliomas and medulloblastomas – brain tumours arising from different regions and lineages – suggested that this antigen may mark a common initiating cell that functions early in the oncogenic process. On the other hand, CD133 is also a fairly promiscuous marker that exists on both normal and neoplastic stem-like cells of many lineages, including haematopoietic and other non-neural lineages and hence may simply be a marker for cells with primordial characteristics.[15] Nevertheless, the emerging message is that an understanding of the biology and management of brain tumours as well as other malignancies may fruitfully be linked to a better understanding of stem cell biology[16,17] – not only for discerning how neoplastic stem cells emerge, behave, and may be targeted, but also for determining how normal stem cells might most effectively be harnessed and exploited to destroy their 'evil twins'.

To be sure, the study and management of brain tumours has benefited of late from important technological advances. For example, the generation of more representative in vivo animal models has helped to accelerate the development of new diagnostic technologies and novel therapeutic agents.[18,19] Surgical techniques have advanced due to improved understanding of anatomy and more precise localisation of tumour and normal tissue[20–22] Oncological neurosurgery is being transformed by advances in imaging technologies, including high-resolution magnetic resonance imaging (MRI), MR spectroscopy and positron emission tomography (PET) scans, as well as diffusion and perfusion imaging which permit better localisation and characterisation of lesions and their relationship with normal brain architecture.[23] Frameless stereotaxis and intra-operative MRI translate imaging details onto the operative field, allowing for precise yet aggressive surgical resection of the lesion.[24] Improvement in intra-operative neurophysiological monitoring as well as increased adoption of awake craniotomy permits the ultimate monitoring of the patient's neurological status during tumour resection.[25,26] While better technology may contribute to improved patient survival, ultimately it will be better molecular and cellular targeting – based on insights into tumour biology – that has the greatest impact. The overall outcome in patients with glioma remains poor due to the intrinsic biological nature of the lesion.[27,28] Median survival for malignant glioma or glioblastoma multiforme (GBM) remains 1 year or less and, for the intermediate grade anaplastic astrocytoma, around 3 years.[29] Glioma cells have the intrinsic capability to infiltrate local structures and to migrate over great distances, leading to disease recurrence despite aggressive resection.[30,31] Scherer originally reported the observation that glioma cells are found to infiltrate and migrate along perivascular, perineuronal, subpial spaces, as well as along white matter tracts such as the corpus callosum.[30] As noted above, it was the startling resemblance of many of these properties to the attributes of the relatively newly-recognised neural stem cell a decade ago that helped trigger the dual interest of not only arming stem cells to track and eradicate tumours but also to view stem cell biology as somehow integral to the emergence and/or propagation of the tumour itself.[1,32,33] It is these aspects that will be reviewed and discussed in the remainder of this review.

2. Neural stem cells

NSCs – as rigorously defined[34] – are multipotent cells with the ability to self-renew and to generate mature, differentiated daughter cells of all neural lineages throughout the developing neuraxis (including neurones of multiple subtypes, astrocytes and oligodendrocytes) as well as to reconstitute those cell types in ablated neural regions.[34–38] Given that their teleology is not only to participate in organogenesis but also to maintain homeostasis throughout life, the NSC is endowed with an intrinsic plasticity that allows it to shift its fate dynamically in response to cues from the local micro-environment in vivo (a process we attempt to emulate experimentally in the laboratory dish by the addition of exogenous molecules). The progeny of NSCs can integrate seamlessly and functionally into the surrounding host neural structures,[35,39–44] making them appealing candidates for nervous system repair. Transplanted exogenous NSCs appear to emulate the behaviour of endogenous NSCs,[45] but with the advantage of being capable of expansion to any quantity desired and of being delivered precisely to the locations and at the times dictated by the experimenter or practitioner.[152] Engrafted exogenous NSCs manipulated ex vivo to express a variety of transgenes can integrate locally at the site of implantation and, if applied to the proper germinal zone, be exploited to disseminate therapeutic genes or to yield some desired neural cell types globally throughout the CNS.[39,40,44,46,47] The tremendous migratory capability of NSCs in conjunction with their innate tropism for intracranial pathologies make them ideal therapeutic agents in a variety of neurological diseases.[48–53] Potential sources of NSCs have been reviewed recently.[54] NSCs, including those of human origin, may be derived directly from neuroectodermal structures or secondarily from embryonic stem cells (ESCs) when subjected to appropriate stimuli in vitro.[55] Both sources of NSCs yield neural precursor cells that can integrate and respond appropriately to developmental cues, including, for example, migrating along established intracranial pathways and differentiating into functional neurones and glia appropriate to the region,[39–41,44,48,56,57] emulating some of the events of early mammalian neural development. It is these behaviours that have suggested the potential of such cells for 'recapitulating development' in the 'post-developmental' brain, when one would like to replace injured or degenerating neural cells.[58] While there is a great deal of debate as to the best source for neural progenitors, it is the behaviour of the NSC derived directly from the neuroectoderm[46,59] which has

established the 'gold standard' for what can and should be achievable by cells with normal stem-like attributes.

3. What is known about NSCs as they relate to brain tumours?

The potential for harnessing stem cell biology against malignancies in general and brain tumours in particular has garnered a great deal of attention of late – particularly because approaching high-grade intracerebral malignancies has begun to be identified as potentially 'low-hanging fruit' for the stem cell therapy field.[33,61–64,153]

3.1. *Exogenous neural stem cells respond to gliomas*

Aboody and colleagues first described that modified NSCs introduced into the parenchyma of not only the ipsilateral but also the contralateral hemisphere, or the cerebral ventricles, as well as the systemic circulation, could migrate over great distances to sites of intracranial pathology, as modelled by a glioma in rodent hosts.[1] Even more surprising, but of great appeal, was the observation that NSCs could and would position themselves in direct juxtaposition to glioma cells migrating away from the tumour bulk to invade normal tissue. This ability to track invading tumour cells signified a potentially powerful way to treat a phenomenon notorious to primary gliomas, which has made their management so vexing. The same group observed reduction of tumour bulk and improved host survival with the use of genetically modified NSCs. A second study, published at the same time, confirmed these findings.[65] Combinations of NSCs from different sources have been shown to exhibit the same gliomatropic effect in experimental rodent brain tumour models and effectively to reduce tumour bulk and prolong host survival.[66–69] Cytotoxicity toward glioma cells is enhanced by a bystander effect when NSCs are armed with suicide genes.[70]

3.2. *Endogenous neural stem cells respond to gliomas*

The presence of endogenous NSCs in the adult mammalian brain is now well accepted. They have been isolated not only from foetal human brains,[48] but also from adult human brains,[59,71–75] often confirmed in neurosurgical specimens. Whether these cells can respond to intracerebral malignancies is an interesting question; it has been proposed that they may contribute to the cellular heterogeneity of the tumour microenvironment.[76] In fact, Glass and colleagues recently demonstrated that endogenous neural precursor cells (NPCs) do show extensive gliomatropism for glioma and migrate from the subventricular zone to surround the tumour graft.[77] Interestingly, the robust migratory response is somewhat abrogated in older mice. Translated into human subjects, this has significant clinical implications, since the incidence of GBM is much higher in elderly patients. More importantly, co-injection of NPCs and GBM leads to apoptosis of glioma cells. Previously published reports have also highlighted the tumour inhibitory effect exhibited by NSCs.[78,79]

Why do not endogenous NSCs efficiently handle intracerebral malignancies? This question is pertinent to all injuries or abnormalities within the nervous system: why does not the endogenous pool of NSCs effectively reverse pathology? Whether it is an issue of pure numerical advantage of the rapidly dividing tumour cells overwhelming the small numbers of endogenous NSCs or other unidentified factors remains to be determined. Or perhaps some 'rogue' endogenous NSCs themselves, under some circumstances, are the culprits giving rise to the tumour from the onset. These are extremely important clinically relevant questions that might influence what type of new chemotherapeutic interventions should be devised – drugs that boost the response of endogenous NSCs to tumours or perhaps those that eliminate some of those NSCs.

4. Molecular breadcrumbs

That NSCs are attracted to regions of intracranial pathology has come to be recognised following numerous studies[32,41,80–82] and has provided much of the impetus for using NSCs to treat various types of intracerebral lesions. Some of the factors proposed to be responsible for drawing NSCs to intracranial pathology, including tumours, have been discussed previously.[33,63] Refinement of genetic techniques will help to identify even more of the molecules that govern such NSC homing in the adult CNS.[83]

Factors released and expressed by the glioma cells, by the tumour stroma (composed of adjacent reactive astrocytes, microglia, oligodendrocytes), by tumour-derived endothelium, as well as the damaged surrounding normal brain itself all contribute to NSC gliomatropism. Some of these agents, such as stem cell factor (SCF) and monocyte chemo-attractant protein-1 (MCP1), have been identified,[84–86,154] yet others are still to be characterised and their role in NSC gliomatropism defined.[87] Chemokines are fundamental to normal development and the proper functioning of various biological systems, particularly in the CNS, and their expression may be deranged in disease conditions.[88] The expression of the heretofore traditionally-viewed haematopoietic chemokine receptor CXCR4 by human and mouse NSCs while its cognate ligand SDF-1α is expressed within regions of CNS injury and degeneration (particularly by reactive astrocytes and endothelium) first suggested that products of inflammation might, in addition to being inimical, also provide the basis for 'calling in' reparative cells, a 'beacon' guiding and directing the homing of NSCs to regions-in-need.[89] The same binding partners are also important in normal CNS development,[90] suggesting that NSC homing to pathology in the 'post-developmental' brain may simply be a recapitulation of the NSC migration that lead to the formation of lamination during CNS organogenesis. Especially relevant to this review, and reinforcing the similarity between normal NSCs and neoplastic cells of the CNS, is the role of CXCR4 and SDF-1α in glioma growth, migration, and tumour angiogenesis.[91,92] Expression of SDF-1α on tumour-associated endothelium while CXCR4 is present on GBM and medulloblastomas suggests a potential paracrine growth loop and a molecular explanation for angiocentric growth by intracranial neoplasms. Use of small molecule antagonists of CXCR4 inhibits the growth of GBM and medulloblastoma in experimental models.[93] Expression of SDF-1α by tumour-derived endothelium serves to attract the migration of NSCs.[94,95] Blocking

SDF-1α/CXCR4 interactions also prevents gliomatropic migration of NSCs.[96] SDF-1α/CXCR4 interactions appear to be pivotal as well to the gliomatropism of circulating adult haematopoietic progenitor cells.[97] Metastatic tumours represent a substantial proportion of intracerebral malignancies. That the receptor CXCR4 is also expressed by metastatic breast cancer cells while its ligand SDF-1α is present on brain endothelium could also provide a basis for the transmigration of circulating tumour cells through the blood brain barrier[98] – another quality shared by stem cells and neoplastic cells.[1,99] Indeed, inhibition of CXCR4 prevented breast cancer metastasis.[100] A recent study suggested a role for vascular endothelial growth factor (VEGF) in mediating NSC gliomatropism.[101] This is especially relevant since VEGF, along with other pro-angiogenic factors, is crucial in maintaining the aggressive behaviour of GBM.[102]

Over-expression of epidermal growth factor (EGF) and constitutive EGF receptor signalling are important events in malignant transformation of gliomas.[103,104] Signalling events downstream from the activated EGFR are also responsible for enhanced invasion and migration.[105] NSC migration is also mediated by the same receptor.[106] Relative concentration gradients in tumour microenvironment could mediate NSC migration toward the EGF source-location of brain tumour cells. The extreme mobility of malignant glioma cells is dependent on the expression of specific gene sets.[107,108] Could glioma cells 'outrun' NSCs such that the response of endogenous NSCs to glioma will inevitably be ineffective? Understanding the role of these genes and subsequent downstream signalling events will help in designing therapeutic agents and appropriately arming *exogenous* NSCs to compensate for and/or target these pathways, thus 'speeding-up' their migration.

5. NSC 'payloads'

5.1. *Therapeutic packages*

Exploiting the unique tropism of NSCs for gliomas, several groups have now confirmed the therapeutic efficacy of using of genetically-armed NSCs to target neoplasms in vivo in a variety of murine brain tumour models, through the delivery of a variety of growth-regulating and anti-glioma gene products (Table 1). Recently, one group demonstrated therapeutic efficacy in the delivery of an inhibitory protein of glioma proliferation and migration in a mouse model.[155] Previously, many of these were introduced into the tumour bulk directly via injection of genetically modified viral vectors.[109–112] Some examples include the introduction of adeno-associated virus (AAV) engineered to express the anti-angiogenic protein angiostatin directly into the tumour.[113] Others have targeted cell cycle pathways in glioma to effect growth arrest.[114,115] Several groups have demonstrated the utility of oncolytic viruses, some of which display unique selective toxicity against cells with specific oncogenic mutations, in a variety of experimental cancer models.[109,116]

NSCs have the unique advantage of behaving as gliomatropic mobile translational factories. Their ability to home in to tumour cells over great distances, the fact that they already possess the full complement of transcriptional, translational, and post-translational capability, and their capacity to convey large amounts of genetic information (beyond the limits imposed by the relatively small genome of the viral vectors), make NSC a much more powerful and adaptive anti-tumour agent.

Aboody and colleagues demonstrated in vivo efficacy of murine NSCs transduced with the gene for the enzyme cytosine deaminase (CDA).[1] The enzyme converts the non-toxic pro-drug 5-fluorocytosine (5-FC) into the nucleoside analogue 5-fluorouracil (5-FU), which is then incorporated into the DNA of the neoplastic cell causing chain termination and cell death. Tumour-bearing mice inoculated with CDA-expressing NSCs and given 5-FC demonstrated dramatic reduction in the intracranial tumour burden. This finding was subsequently corroborated in a different murine tumour model using a different NSC line.[68] The ability for long distance migration towards the tumour bulk, to distribute itself throughout the tumour bulk, and to track micro-deposits of glioma cells escaping from the tumour bulk (juxtaposed to those individual invading neoplastic cells) ensures high concentrations of the pro-drug-converting enzyme (or probably any anti-neoplastic

Table 1 – Strategies in neural stem cell (NSC)-mediated brain tumour therapeutics

	Immunomodulatory	Growth regulatory and tumouricidal	Viral therapy	Pro-drug converting enzyme/suicide gene therapy	Biophysical agents
	Initiation of enhanced anti-tumour immune response via local delivery and expression of high concentrations of cytokines	Induction of tumour growth arrest via interaction with NSCs or binding to expressed growth regulatory factors	Introduction of virus into vicinity of tumour cells causing cytolysis	Enzymatic conversion of pro-drug into toxins. Cytotoxicity is amplified by the 'by-stander' effect	Delivery of agents to vicinity of tumour, which requires subsequent secondary activation
Published studies[b]	IL4, 12 IL2[a]	TRAIL IFN-β	HSV Adenovirus AAV	Cytosine deaminase HSV-TK	
Future?	GM-CSF	PF4 (platelet factor 4) TNF(73)	Reovirus type 3 VSV	Deoxycytidine kinase	Nanoshells photodynamic therapy

IL, interleukin; GM-CSF, Granulocyte-macrophage colony stimulating factor; AAV, adeno-associated virus; HSV-TK, Herpes simplex virus thymidine kinase; VSV, Vesicular stomatitis virus; TNF, tumour necrosis factor; IFN, interferon.

a Transduced MSCs.

b Refer to text for references.

gene product of choice) in the region of the tumour cells. Systemic administration of the pro-drug reaches the CNS and is activated by the converting enzyme in the engineered NSC in close proximity to the tumour cells. The CDA pro-drug system, in particular, engenders an extremely large 'bystander effect', i.e. the killing of even a small number of tumour cells sends 'ripples' of oncolytic factors emanating from that epicentre of cell death to kill an even broader region of tumour cells. Therefore, even if the CDA transgene were to be down-regulated in some NSCs, the oncolytic action of the population of NSCs would probably remain undiminished. Furthermore, while evidence suggests that the exogenous NSCs never contribute to or exacerbate the tumour mass or become oncogenic themselves,[117] should such an untoward eventuality emerge, CDA would serve as a suicide gene within the NSC – hence a built-in safety mechanism.[70] Other pro-drug systems exist – some have been tried successfully in NSCs (e.g., herpes simplex virus (HSV) thymidine kinase (TK))[117] while others (e.g., deoxycytidine kinase)[118] have yet to be tested using NSCs.

Another approach utilises NSCs as engraftable, mobile, gliomatropic viral packaging lines.[119] In the past, the effectiveness of viral-mediated gene delivery to aggressively virulent brain tumours was limited by the 'halo' effect, i.e. only tumour cells within a limited radius of injected viral vector were eradicated; tumour cells situated beyond that radius could escape to set up new satellite tumours. However, by using a migratory gliomatropic stem cell itself to produce and deliver the viral particles to widely dispersed tumour cells, viral vector-mediated dissemination of the oncolytic gene could be enhanced, hence overcoming this previous treatment limitation. One study reported effective killing of tumour and escaping micro-deposits in a murine host by using murine NSCs to release replication-conditional HSV TK,[117] hence overcoming the typical low transduction frequency encountered in HSV glioma gene therapy by directing delivery of the virus to the intended cellular targets. This targeted cytotoxic effect, mediated by conversion of the pro-drug ganciclovir by TK into ganciclovir phosphate, is greatly amplified by virtue of the 'bystander effect'.[70,60] As noted above, the bystander effect increases the killing of surrounding tumour cells not in physical proximity to the genetically engineered NSCs, a mechanism probably mediated by expression of connexin-43 in untransduced glioma cells.[69] A recent study demonstrated a similar efficacy for gliomatropic NSCs engineered to be a viral packaging cell line for adenoviral-based vectors.[120] In short, NSCs, acting as mobile factories for engineered viral vectors and suicide genes, seem to have significant advantages over the injection of viral supernatants alone or the use of immobile fibroblast viral packaging cell lines.

The above-described protocols rely on the generation of cytotoxic agents that are deadly not only to tumour cells but also to dividing NSCs.[70] On one level, as noted above, this fact provides a measure of security against the possibility (albeit unlikely and never observed) that the NSCs themselves could become tumourigenic. NSCs that are quiescent or that have differentiated into mature neurones and glia would be spared. Nevertheless, this mechanism illustrates the importance of carefully co-ordinating NSC implantation and administration of the non-toxic pro-drug such that the NSCs have adequate time to infiltrate the tumour and seek out escaping glioma cells. Having the ability to track the migratory progress of the engineered therapeutic NSCs should help in this regard (see the following section).

Immunotherapy of brain tumours via the direct instillation of cytokines or via the use of genetically modified viral vectors represents another therapeutic strategy.[121–123] Cytokines have been used successfully in other types of human malignancies.[124,125] Some of these directly effect tumour toxicity, while others force growth arrest and differentiation. Another class of cytokines modulates the host immune system to initiate an anti-tumour response. Benedetti and colleagues reported that gliomotropic NSCs transduced with a gene encoding the immunomodulatory cytokine interleukin-4 (IL-4) prolonged the survival of brain tumour bearing mice.[65] The NSCs persisted several weeks post-injection. The authors made another interesting observation: even NSCs not transduced with the IL-4 gene had a tumour-inhibitory effect, suggesting that NSCs themselves might have an intrinsic oncostatic action. This inherent anti-tumour activity by NSCs (clone C17.2) was first reported 15 years ago[78] and was corroborated recently by another group using a different biological system.[79]

Ehtesham and colleagues showed that murine neural progenitors transfected with immunomodulatory cytokine IL-12 gene using an adenoviral vector are capable of stably expressing IL-12 in vivo, reducing the tumour burden, and improving survival upon implantation into brain tumour-bearing syngeneic mice.[66] The authors also observed infiltration of the tumour by T lymphocytes in response to regional expression of IL-12; a finding corroborated by others.[126] NSCs overcome the hurdle of achieving a high enough local concentration of therapeutic compounds by their specific homing ability. This is again exploited in the local delivery of TRAIL (*T*umour necrosis factor-*R*elated *A*poptosis-*I*nducing *L*igand), a pro-apoptotic protein belonging to the tumour necrosis factor superfamily which has been shown to induce tumour apoptosis in experimental models.[127] Implantation of TRAIL-expressing NSCs into nude mice bearing GBM xenografts was followed by tumour apoptosis and reduction.[67] Similar efficacy was seen using NSCs of human origin. Using an adenoviral vector to transduce the TRAIL gene into NSCs isolated from the telencephalon of a human foetal cadaver (13 week gestation),[128] Kim and colleagues reported the apoptosis of human GBM cell lines in vitro and extensive gliomatropic migration in vivo in brain tumour-bearing nude mice, resulting in reduction of intracranial tumour burden.

Because one potential strategy for tumour eradication is the mobilisation of the patient's own immune system against the cancer, it is intriguing to contemplate the notion that the NSCs used need not be matched to the donor. In other words, a universal readily available, abundant, well-characterised NSC cell line for use as an off-the-shelf therapeutic reagent by all patients is feasible. Should these rapidly tumour-infiltrating NSCs provoke an immunological response directed against themselves, so much the better for tumour elimination.

Malignant gliomas express a variety of pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), which makes them

one of the most angiogenic of all solid tumours.[129–131] In fact, grading of malignancy is reliant on the presence of tumour vascular proliferation.[132] Application of anti-angiogenic compounds, therefore, holds potential for anti-glioma therapy. However, some of these molecules have poor intracranial bioavailability.[113,133] Endostatin, for example, a potent anti-angiogenic compound, has a short half-life in vivo and bioavailability is restricted by the blood–brain barrier. It is this type of therapeutic challenge, i.e. adequate delivery to the CNS, for which NSCs as delivery vehicles are ideally suited. While encapsulation of cells engineered to express endostatin in alginate gel beads can prolong the intracranial bioavailability of endostatin,[134] one would anticipate that transfection of engraftable NSCs with the genes encoding endostatin or anti-angiogenic molecules would be the most effective strategy for achieving high local concentrations of these molecules. The delivery of such molecules preferentially to vascular endothelium within the tumours is further supported by the observation that NSCs have a predilection for, and transmigrate through, tumour endothelium via association of their CXCR4 receptors with endothelial-expressed SDF-1α, as well as via α4-integrin.[94] These endothelial factors, along with other trophic factors released by the tumour, and the local microenvironment help to attract the modified NSCs to the target cells.

It should be noted that, although the tropism of stem cells for cancer was first unveiled by observing the behaviour of NSCs, this action might not be limited to only stem cells of neuroectodermal origin or to CNS tumours.[135] Indeed, bone marrow mesenchymal stem cells (BMSCs) engineered to express interferon-β (IFN-β), when injected into the carotid artery of brain tumour-bearing mice, also appeared to migrate in a gliomatropic fashion and destroyed the tumour via direct cytotoxicity, resulting in prolonged survival of the hosts.[136] Others have shown that BMSCs overexpressing the immunomodulatory cytokine IL-2 migrate to the contralateral tumour-bearing hemisphere via the corpus callosum helping to promote tumour destruction.[137] Furthermore, BMSCs administered systemically appeared to localise to prostate and breast cancers metastatic within the periphery. As to which type of stem cell is bested suited for which type of tumour within which region will need to be determined empirically. At present, we favour the view that stem cells derived from the lineage-of-origin of the cancer are best suited for 'hunting it down' and eradicating it.

5.2. *Diagnostic packages*

Diagnostic imaging is another important facet of neuro-oncology that might be addressed by NSCs. Given that NSCs appear to be capable of crossing the blood–brain barrier and of finding even small micro-deposits of tumour cells, they might be 'tagged' to serve as smart and mobile imaging agents.[138] Similar technology is being used to track in vivo NSC migration in rodent brains in response to ischaemia and immune response.[139] They clearly would have significant advantages over traditional 'dumb' radiographic contrast agents, such as gadolinium, which depends on endothelial incompetence in neoangiogenic vessels. Tang and colleagues showed persistence of firefly luciferase-expressing murine NSCs (clone C17.2) up to 4 weeks in living mice, as demonstrated by routine bioluminescence imaging.[140] This powerful technique can be exploited to track the migration of specially tagged NSCs in living hosts. NPCs have been co-transduced with both soluble-TRAIL (s-TRAIL) and luciferase to permit evaluation of in vivo migration and the delivery of tumouricidal s-TRAIL to the tumour cells.[141]

A different imaging modality exploits the exquisite sensitivity of magnetic resonance imaging (MRI) to ferromagnetic nanoparticles, which are conjugated via tat peptide-derivitisation.[142] This methodology was later expanded using more advanced MRI imaging protocols, which nicely demonstrated dynamic gliomatropic migration of NSCs and MSCs introduced into the tail vein and the cisterna magna.[143] The authors were able to track the movement of as few as 1000 labelled NSCs in living rodents using MRI. Anderson and colleagues visualised the migration and incorporation of labelled bone marrow-derived endothelial precursor cells into brain tumour-associated neovasculature.[144] In addition, these cells could function as gene delivery vehicles to the vasculature. The simple incubation with or lipofection of superparamagnetic iron oxide particles was sufficient to label human haematopoietic progenitor cells that could be tracked in vivo in living murine hosts.[145]

Over the past several years semiconductor quantum dots have emerged as a promising experimental and clinical imaging agent.[146] Several groups have demonstrated their utility for the whole-body in vivo tracking of labelled cells using wavelength-resolved spectral imaging.[147,148] Labelling and in vivo monitoring of NSCs should be achievable using similar protocols. Metal nanoshells represent a new generation of clinically promising nanoparticles with both diagnostic and therapeutic uses.[149] Selective tunability permits strong absorption of near infrared (NIR) light, which also has optimal tissue penetration resulting in thermal activation and ablation of tissue in close proximity to the target tissue. In addition, the particles are MRI visible, allowing for real-time tracking.[150] One can foresee the use of NSCs as carriers of nanoshells for targeted delivery to intracranial tumour bulk and escaping microdeposits. NIR activation would result in thermal ablation of both the NSCs as well as the tumour cells.

6. A future scenario

Consider the following hypothetical clinical scenario perhaps a half decade from now: a woman in her 50s presents with headache and right-sided weakness. On physical examination she has a right hemiparesis and florid papilloedema consistent with elevated intracranial pressure. An MRI shows a heterogeneously enhancing lesion in the left frontal lobe with associated oedema and mass effect. There are also signal abnormalities involving the genu of the corpus callosum and the contralateral frontal lobe, suggestive of diffuse and distant tumour involvement. The imaging characteristics as well as subsequent magnetic resonance (MR) spectroscopy are highly suggestive for a high-grade glioma. NSCs are obtained from either stable cell lines or generated from human embryonic stem cells induced in vitro to stably develop into NSCs. The NSCs are expanded ex vivo and transfected with genes encoding for growth regulatory proteins and for anti-tumour factors, as well as for smart 'bioimaging' labels. The

transfected cell 'cocktail' is then seeded onto a biopolymer matrix and grown for several days in the presence of growth factors. The patient undergoes surgical debulking of the tumour with the help of frameless stereotaxis followed by implantation of the biopolymer matrix loaded with 'therapeutic' NSCs into the resection cavity. This is somewhat analogous to the placement of drug-impregnated wafers or brachytherapy radioactive seeds into the post-tumour resection cavity except NSCs represent 'smart' homing delivery vehicles that are highly mobile and have the unique ability to home into escaping glioma cells as well as tumour-associated endothelium (Fig. 1a and b). Transduction of the NSCs with smart-imaging agents helps the patient's physicians to track the migration of the NSCs as well as detect distant deposits of escaping glioma cells. In addition, the NSCs have been transduced with differential sensors that activate the transcription of unique biomarkers detectable externally. For example, MRI in conjunction with bioluminescence is used not only to track the migration of the NSCs, but also to indicate the presence or absence of residual glioma cells.

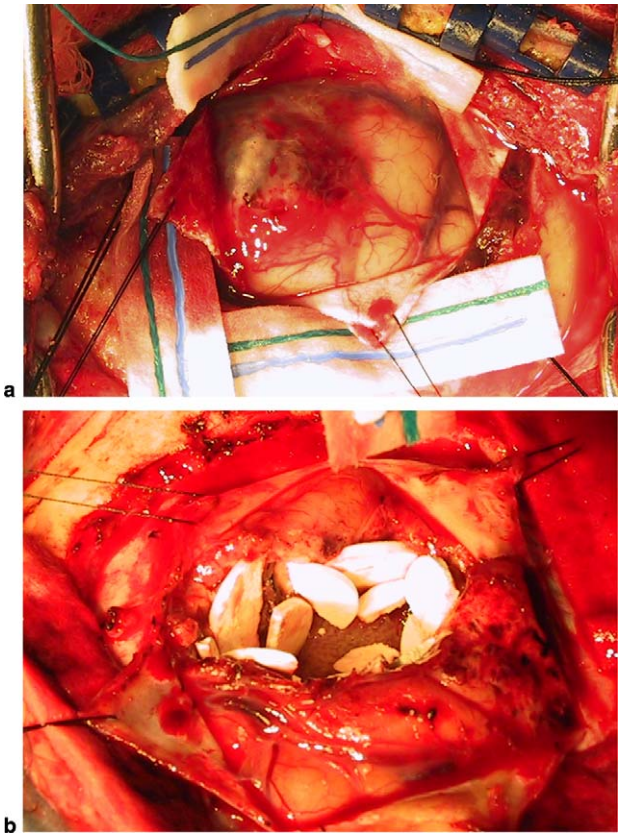

Fig. 1 – (a) High-grade glioma at time of resection. The tumour is grossly involving the superficial cortex. (b) Post-resection cavity of an anaplastic oligo-astrocytoma. The tumour recurred despite two previous surgical resections, systemic chemotherapy and external beam radiotherapy. Resection of the recurred tumour is followed by placement of biopolymer wafers laden with the chemotherapeutic agent BCNU or Carmustine. In the future genetically-engineered neural stem cells could potentially be used in its place as a "smart" and adaptive biotherapeutic tool.

These marker NSCs will become useful later on, in helping to detect possible tumour recurrence (as distinguished from radiation necrosis). Molecular determination of the gene expression profile and cancer genomics, in conjunction with examination of the histopathology of the resected specimen, helps accurately to classify the tumour, provide a prognosis, and tailor additional therapy. If recurrences become evident, NSCs (perhaps engineered to target a different molecular pathway) can be re-administered either into the ventricles (a simple surgical procedure) or into a biopsy cavity or both. NSCs that have been administered via the biopolymer matrix, acting as a scaffold for the exogenous NSCs and their cellular processes, aid in subsequent inhibition of inflammation and scarring, and perhaps in helping to promote a degree of reconstitution of the injured parenchyma by facilitating physical interaction with endogenous neurones, glia, endothelium, and progenitors and catalysing their intrinsic regenerative actions.

Obviously, a great deal of biology – stem cell biology, tumour pathophysiology, molecular imaging, gene regulation – must be learned before the above becomes an actual clinical scenario. Safety must also be rigorously affirmed. However, as described in this review, some pieces of the puzzle are already falling into place. Significantly, brain tumours may represent 'low-hanging fruit' for the stem cell field, not only because this disease has such a dismal outcome with no near-term practical therapies in the offing, but because the known biology of the stem cell matches well some of the known inadequacies of present interventions – the inability to attack aggressively invasive tumour cells. Furthermore, not much is actually being 'asked' of the stem cell but to 'find' the tumour cells, deliver its payload, and 'not cause any mischief'. The stem cell need not differentiate into a particular neural cell type nor make specific connections. It need not even persist. On the other hand, in approaching this disease, practical protocols for preparing and delivering stem cells will be devised, safety will be proven, the behaviour of stem cells in a human brain will be observed, lessons will be learned, and a much needed early success (measured by life extension of even a few months) will be garnered for the stem cell field. It is important to see NSCs (delivered by minimally invasive techniques) as a component of a multifaceted approach to the management of intracranial tumours and not as a replacement or substitute for other important approaches.[151]

Conflict of interest statement

None declared.

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