Stem cells and models of astrocytomas

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Abstract

Purpose: To provide a critical assessment of current stem-cell based pre-clinical models of astrocytomas (gliomas).

Methods: Data were archived from MEDLINE using Boolean formatted keyword queries. Top articles were selected for critical analyses depending on the qualitative assessment of the citation index, novelty of the findings, reputation of the research group and relevance to stem-cell based pre-clinical models of astrocytomas.

Results: The emergence of stem-cell based pre-clinical models of gliomas offers advantages for cellular transformation studies over other current *in-vitro* cell cultured based models. Cells utilized in these stem-cell based pre-clinical models are easier to transform, with the induced tumours demonstrating very high molecular and pathological recapitulations of astrocytomas that are derived from humans. These stem-cell based models fall into two categories. In the first, synthetic astrocytes can be differentiated from various stem cell sources such as the nervous system and embryos, and utilized in elegant forward genetic strategies to develop novel astrocytoma pre-clinical models. The second category represents a cancer stem cell pre-clinical model. In this model, glioma stem cells exhibit very high pathological recapitulations of the human tumours, and can be very informative to comprehend the basis of radio-chemoresistance among patients.

Conclusion: The quest to develop robust pre-clinical models of astrocytomas is on an ongoing basis. The models are of clinical importance for the discovery of effective treatment modalities that can considerably improve the health of patients with this deadly disease.

Primary brain tumours are among the top 5 causes of cancer-related deaths in the adult and pediatric populations¹, with gliomas accounting for the majority of cases². Astrocytomas are the most common sub-type of glioma, with four pathological grades as classified by the World Health Organization (WHO) (Figure 1)³. Glioblastoma multiforme (GBM) is the most common malignant astrocytoma, which despite current therapy (surgery, radiation and chemotherapy), has a median survival of about 1 year.⁴ Although pathologically indistinguishable, there are two subtypes of GBMs. Primary GBMs (60% of subjects > 50 yr) arise rapidly or *de novo* without any previous clinical or histopathological history, whereas secondary GBMs (40% of subjects < 45 yr) derive from preexisting low-grade lesions after long latency ranging from 5 to 10 yr (Kleihues and Cavenee, 2000), both of which correspond to at least two genetic progression paradigms (Figure 2).^{3,5,6} These sub-types of GBMs also have contrasting responses to current therapies, most likely as a consequence of differences in the genetic pathogenesis.

Overall, astrocytomas like other tumours are molecularly heterogeneous with numerous genetic alterations, including single gene defects and chromosome aberrations that are of epidemiological, prognostic and therapeutic relevance. Common to all astrocy-

A

Astrocytic tumors	WHO classification
Pilocytic astrocytomas	I
Pleomorphic xanthoastrocytomas	I-III
Subependymal giant cell astrocytomas	I
Diffuse astrocytomas	II
Fibrillary	
Protoplasmic	
Geministocytic	
Anaplastic astrocytomas	III
Glioblastoma	IV
Giant cell sarcoma	
Gliosarcoma	

B

FIGURE 1. Classifications of astrocytic tumors according to the World Health Organization (WHO). (A) Pathological variations of astrocytomas within each gradations. (B) Pathological examples of each gradations of astrocytomas. Grade I astrocytomas are usually indolent with limited invasive potentiation. Some tumors may demonstrate microvascular pleomorphism and cellular pleomorphism. Grade II astrocytomas have cellular pleomorphism and limited invasiveness. Grade III astrocytomas have a profound increase in mitotic index, moderate degree of cellular pleomorphism and nuclear atypia. Grade IV astrocytomas demonstrate pathological features of Grade III astrocytomas in conjunction with necrotic sites (asterisks) and microvascular hyperplasia (arrows). Of note, sample pictures of each gradation of astrocytomas are taken from N Engl J Med 2005 353(8):811-22, with permission.

tomas are aberrations in cell-cycle regulation, mediated by both p53 and pRb regulated pathways, plus several aberrant growth factor-receptor-downstream signaling pathways.⁷ As part of the ongoing Cancer Genome Project and numerous independent international groups, our understanding of the genetic basis of astrocytomas has been significantly enlightened.⁹⁻¹⁴ For instance, over 220 regions of the human genome are discovered with a gain or loss of

genetic content in patients with primary or secondary glioblastoma multiforme¹⁴.

In an effort to eradicate this deadly disease among patients, there is a continual trend towards the development of robust pre-clinical models of astrocytomas. An ideal pre-clinical model must conservatively recapitulate both the pathological and molecular properties inherent in the patients' tumours. The model must also be amenable towards therapeutic response studies in

FIGURE 2. The genetic pathogenesis of glioblastoma multiforme. Primary glioblastoma arises from a direct transformation of a glioma precursor cell which typically involves concurrent mutations in several genes such as EGFR, VEGFR, and so on. Secondary glioblastoma arises from a progressive series of pathological events with each progression step associated with a distinct cocktail of genetic mutations. Of note, both types of glioblastomas are associated with shared and with distinct genetic signatures.

our effort to discover treatments with outstanding efficacies. Recent discoveries have prompted more of a focus on the stem cell, progenitor cell and early-differentiated cell type lineages in the origin of astrocytomas, which is of therapeutic relevance. This article describes some of the current efforts that are established to develop novel pre-clinical models of astrocytomas, by specifically addressing whether these early lineage cell types can be implemented in the development and therapeutic applications of robust models. At this time, it is important for physicians to become

acquainted with the prospects of these novel stem-cell based pre-clinical models, which are anticipated to revolutionize the way of how we understand the biology of astrocytomas. This body of knowledge will inevitably be of clinical importance in devising effective therapeutic modalities, as an advancement towards the betterment in the treatment and care of these terminally ill patients.

Methods

Data were archived from MEDLINE, using Boolean formatted queries on the keywords including: glioma stem cells, stem cells, astrocytoma, glioblastoma multiforme, astrocytes, synthetic astrocytes, progenitor cells, ENU, MNU, viral, brain tumours and brain tumour model. Only the top articles were selected for critical analyses depending on the qualitative assessment of the citation index, novelty of the findings, reputation of the research group and relevance to stem-cell based pre-clinical models of astrocytomas.

Results

Current perspectives on the origin of astrocytomas

An understanding of the origin and progression of astrocytomas is pertinent towards the eradication of this incurable disease. In fact, both the assortment of specific genetic mutations and cell types contribute towards the origin of gliomas. Mature astrocytes can de-differentiate and transform into astrocytomas depending on the type of mutation. For instance, an increase in the expression of the Platelet Derived Growth Factor Receptor-Beta gene can de-differentiate and transform mature astrocytes.¹⁵ However, the major pitfall of this postulate is the inability to explain the origin of tumours with mixed ontogenies.

An alternative hypothesis on the origin of astrocytomas is based on the neoplastic transformation of early cell lineage types that have a stem-cell like property.¹⁶ It is not precisely defined what specific cell types contribute towards the stem-cell origin of gliomas. However, Neural stem cells (NSC), glial progenitor cells and early-differentiated/immature glial cells are speculated as being the most likely candidates. Niches for these cell types are in the sub-ventricular zone (SVZ), dentate gyrus, hippocampus and subcortical white matter¹⁶. In fact, astrocytes in the SVZ demonstrate stem-cell like properties¹⁷. These cells share common features such as an enhanced proliferation, high motility, multi-potency, as-

sociation with blood vessels, regulation by many cellular pathways that are evident in brain tumours, and the expression of immature antigenic phenotypes¹⁶.

Animal model studies on the origin of astrocytomas

Early evidence

Many decades ago, preliminary evidence was provided to strengthen the postulate that early cell lineages are implicated in the origin of brain tumours including findings of the sub-ventricular zone as being more sensitive to chemical and viral oncogenesis in comparison to other central nervous system areas harboring a low proportion of proliferating cells.¹⁸⁻²⁰ For instance, in canine and rodent brains, avian sarcoma viral transformation or systemic exposure to the N-ethyl-N-nitrosourea chemical mutagen preferentially succumb to tumor development in the sub-ventricular zone.^{19,20} These preliminary experiments however were uninformative in dissecting the genetic etiology of brain tumours.

Somatic transgenesis evidence

Mouse models of gliomas have been developed such that glioma potentiation can be induced using somatic transgenesis methods. With somatic transgenesis, cocktails of interacting known genes carried in engineered lentiviruses, retroviruses or adenoviruses can be stereotactically delivered to specific regions of the rodent brain to transduce certain cell types. This method has been successful in demonstrating that mutating the neural stem cells, neuro-progenitor cells and early differentiated astrocytes within or juxtaposed to the sub-ventricular zone with elevated expression of oncogenes such as EGFR, AKT and RAS, can induce the development of high grade astrocytomas.²¹⁻²⁴ This is in contrast to the findings of murine mature astrocytes from the outer cortex that are less susceptible to transform when mutant.²¹⁻²⁴ Although this method is very powerful to understand the origin of astrocytomas, the use of lentiviruses and retroviruses can

sometimes mutate endogenous genes of the transduced cells that can potentially affect transformation. Furthermore, adenoviruses are only biased towards transducing cells that only express the CAR receptors.²⁵

Germline transgenesis evidence

Germline transgenesis has been effectively utilized to develop an assortment of Genetically Engineered Mouse (GEM) models of gliomas. However, the pitfall of these mouse models stems from the observation of frequent variable penetrance for tumour development. Despite this, these mouse models have an inherent developmental microenvironment to facilitate an understanding of how specific genes interact, and how stromal cells interact with tumour cells during astrocytomagenesis. For instance, mice with ubiquitous loss of function mutations in the p53 tumour suppressor gene in conjunction with a loss of function mutation in the NF1 tumour suppressor gene only in astroglial cells, can succumb to developing glioblastoma multiforme within 4 months after birth. These tumours originate from the sub-ventricular zone of the cerebrum and express multi-lineage cellular markers, including that of stem cells.²⁶

Cell culture based pre-clinical models of astrocytomas

Somatic Astrocyte cell culture pre-clinical models and pitfalls

As an alternative to the use of glioma cell lines to develop glioma models, somatic astrocytes can be utilized (Table 1). It must be noted that when in-vitro somatic astrocyte cultures are established, the majority of cells that remain in culture are more reminiscent of mature or terminally differentiated astrocytes. To facilitate these mature somatic astrocytes to become conducive to transform, pre-immortalization is pertinent. Pre-immortalization can be achieved either spontaneously or via the constitutive expression of genes such as telomerase to bypass cellular senescence.²⁷⁻²⁹ For instance, when pre-immortalized human mature somatic astrocytes are mutated to express the human papillomavirus 16 E6 and E7 genes in conjunction with the oncogenes RAS and AKT or the GATA6 tumour suppressor gene, de-differentiation and transformation into high grade astrocytomas occurs.²⁷⁻²⁹

The use of somatic astrocytes however has several pitfalls to develop models of gliomas (Table 1). These include difficulty in the establishment and in-vitro propagation while in culture. For instance, human mature astrocytes have a finite lifespan in culture after 20 days upon which entry is into cellular senescence.²⁷ Another pitfall is that there is a paucity of somatic astrocytes that are non-transformed but have mutant genetic backgrounds. Transformation studies warrant multiple mutations to induce full de-differentiation and transformation. In light of these pitfalls, it is therefore imperative to explore alternative in-vitro strategies in the creation of efficient and robust glioma models.

TABLE 1: Pros and Cons of non-stem cell based cell culture models of astrocytomas

	Pros	Cons
Somatic astrocyte cultures	Preserves cellular plus genetic architectures Can be useful for forward genetic studies	Difficult to establish and propagate Senescence is inevitable Not very conducive for transformation studies
Glioma cell lines	Easy to propagate Preserves some genetic plus pathological signatures of the host tumor Preserves some therapeutic response signatures of the host tumour	De-novo cellular, molecular and pathological signatures Cellular heterogeneity can arise Complex genetic signatures

Alternative astrocytoma cell culture based pre-clinical models

Definition of synthetic cell lineages

Stem cells and progenitor/precursor cells can be used to differentiate into multi-lineage cells under various cell-culturing conditions. These cells that are differentiated from stem cells or progenitor/precursor cells need to be designated as being synthetic cell lineages. Many investigators have designated these differentiated cells as an astrocyte, neuron and so on. However, to do so is problematic. The term synthetic cell lineage is more appropriate since these cells are synthesized under user-defined *in-vitro* cell culture conditions and therefore lack the normal developmental and physiological cues that are necessary to develop into somatic astrocytes. Secondly, there is some controversy as to what exactly are these cells that have been differentiated from the stem cell/progenitor/precursor cell sources. For instance, studies on the neurons and astrocytes that are differentiated from skin precursor cells have demonstrated them as being neuron-like or astrocyte-like with the expression of some terminally differentiated markers, however they are in fact non-functional.^{30,31}

Sources of synthetic astrocytes

Synthetic astrocytes have been differentiated under various cell-culturing conditions from numerous stem cell and progenitor/precursor cell sources. These include umbilical hematopoietic stem cells, bone marrow stem cells, neural stem cells, embryonic stem cells, skin precursor cells and glial restricted precursor cells.^{30,32-38} There are a multitude of strategies utilized to synthesize astrocytes under user-defined *in-vitro* conditions. These include treatments of the stem/progenitor/precursor cell sources with retinoic acid³⁹, growing cells on a layer of endothelial cells⁴⁰ or astrocytes⁴¹, growth in the presence of cytokines such as Epidermal growth factor (EGF)⁴², Ciliary neurotrophic factor (CNTF)⁴³, Activin⁴⁴, Fibroblast growth

factors (FGFs)⁴⁵ and Bone Morphogenetic proteins.⁴⁶ However, the final yield of synthetic astrocytes is very variable. The use of a more stringent and step-wise differentiation process can substantially increase the yield of synthetic astrocytes.³³

Usefulness of synthetic astrocytes to develop astrocytoma pre-clinical models

Synthetic astrocytes can be excellent cellular sources in the derivation of robust pre-clinical models of gliomas (Figure 3). They offer the major advantage of having increased genetic and pathological stability in contrary to glioma stem cell pre-clinical models.³³ Of course, it must be recognized that these *in-vitro* based pre-clinical models are challenged by the lack of relevant developmental, epigenetic and micro-environment cues reminiscent in the animal pre-clinical models. Such factors can ultimately influence the genetic, pathological, plus treatment response properties of the induced tumours, and can inevitably challenge the robustness of these *in-vitro* based pre-clinical models. However, since animal pre-clinical models are less efficient and costly, the development of alternative models such as stem-cell based pre-clinical models need to be explored, and can in fact be used to complement the animal pre-clinical models.

Applications of synthetic astrocytes to model astrocytomas

Although synthetic astrocytes were differentiated from many stem cell and progenitor/precursor cell sources in the past³²⁻³⁸, only two sources have been utilized so far in the development of astrocytoma models. These two sources, described below, include both neural stem cells and a non-neural stem cell, namely embryonic stem cells. They have been most useful to comprehend the biology of astrocytomas using elegant forward genetics strategies.

FIGURE 3. An outline of the spectrum of applications that can be associated with the use of synthetic cell lineages, such as synthetic astrocytes. These cellular substrates which are more reminiscent in the origin of astrocytomas can inevitably assist in deciphering the molecular mechanisms of astrocytomagenesis, and to serve in elegant therapeutic design and testing studies.

Source - Neural stem cell (NSC) differentiated synthetic astrocytes

Cellular and transformation properties

Somatic neural stem cells were initially postulated to be prevalent only in the embryonic/fetal brain. However, postnatal and adult neural stem cells are also evident.⁴⁷⁻⁴⁹ Furthermore, synthetic neural stem cells can be differentiated from stem cell sources such as embryonic stem cells and mesenchymal stem cells.^{50,51} In fact, both somatic neural stem cells and synthetic neural stem cells can be differentiated into

synthetic astrocytes under various growth factor treatments⁴⁰⁻⁴⁶, and with varying efficiency. Most remarkable, in a study of synthetic astrocytes differentiated from murine neural stem cells, these synthetic astrocytes are deemed to be most similar to immature somatic astrocytes, and the ability to self-renew with an enhanced proliferation.³² This is in contrast to mature murine somatic astrocytes that enter cellular senescence by the 10th passage upon culturing.³² Neither the synthetic nor somatic murine astrocytes transform *in-vitro* or *in-vivo*. However, these synthetic astrocytes when mutated with a cocktail of glioma genes such as

the p53 tumour suppressor gene and EGFR oncogene, have a higher propensity to transform, than mature murine somatic astrocytes, which do not. In this manner these synthetic astrocytes demonstrate in-vitro anchorage independent growth in culture, and can grow into high grade differentiated gliomas intra-cranially in immuno-deficient mice.³²

Source - Embryonic stem cell (ESC) differentiated synthetic astrocytes

Cellular and transformation properties

Embryonic stem (ES) cells have been proposed for many applications including tissue repair and regeneration, plus embryonic stem cell transgenesis. They can also be differentiated into specific cell types with prospects to understand the process of cell differentiation and gliomagenesis. ES cells were initially established from pluripotent cells that reside in the inner cell mass (epiblast) of murine blastocysts^{52,53} with subsequent isolation from other primate embryos such as the marmoset, cynomolgus and rhesus monkeys, and humans.⁵⁴⁻⁵⁸ They are characterized by the ability to self-renew for indefinite periods and clonogenic property. Under locally controlled conditions comprising of an assortment of growth factors or in-vivo micro-environment cues, these ES cells can differentiate into multiple cell types representative of all three germ layers, including synthetic astrocytes, with demonstrative characteristic morphology and specialized functions.⁵⁹⁻⁶²

Like synthetic astrocytes differentiated from murine neural stem cells, synthetic astrocytes differentiated from embryonic stem cells are most similar to immature somatic astrocytes.³³ These cells can self-renew with an enhanced proliferation and express immature antigenic phenotypes. While embryonic stem cells exhibit anchorage independent growth and can grow into tumours reminiscent of teratomas^{33,54,56}, the synthetic astrocytes do not.³³ Most remarkable, these synthetic astrocytes have a higher propensity to transform when mutated with a cocktail of glioma onco-

genes such as RAS, AKT and MDM2, than murine mature somatic astrocytes, which do not. In this manner, these mutated synthetic astrocytes demonstrate anchorage independent growth and can grow into intra-cranial tumours in immuno-deficient mice, which are predominantly and pathologically similar to undifferentiated high grade gliomas.³³

Pitfalls of using synthetic astrocyte pre-clinical models

Overall, these synthetic astrocytes that are most similar to immature somatic astrocytes seem to be more reminiscent in the origin of gliomas, when mutant.^{32,33} These findings transcend prospects for future research to address the robustness of these models. However, it must be recognized that certain mutations in the stem cell or precursor/progenitor cell source can affect differentiation into a synthetic astrocyte cell lineage without succumbing to transformation into a tumour of astrocytic ontogeny. For instance, the overexpression of Bone Morphogenic Protein (BMP) in transgenic murine neural stem cells induces an increase in astroglial lineage commitment⁶³, while murine embryonic stem cells that are mutant for both copies of the GP130 gene do not differentiate in-vitro at all into synthetic astrocytes from neuroepithelial progenitor cells.⁶⁴ In a similar manner, embryonic stem cells with losses of both copies of the PTEN, PEM or APC genes induce severely perturbed differentiation capabilities.⁶⁵⁻⁶⁸ There are however some mutations such as losses of both copies of the c-FOS gene, or a loss of a single copy of the p53 gene that do not alter the differentiation potential of the stem cell source.^{33,69}

Cancer stem cells as a model for gliomas

The Cancer stem cell hypothesis⁷⁰ has gained momentum within the field of oncology. A cancer stem cell is defined as a cell within a tumour that has the capacity to self-renew and to differentiate into a heterogeneous lineage of cells.⁷⁰ Cancer stem cells have been identi-

fied from many types of tumours including hematological, colon, breast, bone, skin, lung, ovarian and brain.⁷⁰ Neurosphere-like-forming cells, now known as tumourspheres, were isolated from both pediatric and adult gliomas, and even glioma cell lines.⁷¹⁻⁷⁹ Of most clinical importance, glioma stem cells are suspected to be responsible for tumour recurrence and current treatment resistance among patients.⁸⁰⁻⁸³

Cellular properties

Potential sources of glioma stem cells include mutations in neural stem cells and the persistent progenitor astroglial cells of the sub-ventricular zone.⁸⁴⁻⁸⁶ Table 2 summarizes the similarities and differences between a glioma stem cell and a normal neural stem cell. Indeed, a small sub-population of brain tumour stem cells characterized by the expression of the cell surface CD133 protein, and low retention of the Hoechst 33342 dye in the cell nucleus, were isolated from astrocytomas.^{71-77,87} These CD133⁺ cells are in low abundance with an estimated population of ~0.01-5% of the tumour mass, unlike the remaining cells of the tumour mass that are terminally differentiated and cell-death committed. However, there is a positive correlation between the abundance of CD133⁺ cells and the aggressiveness of the astrocytoma.^{71-75,87} These cells form tumourspheres in culture that have about 600-800 adhering cells. The rate of tumoursphere formation is significantly faster compared to neurosphere formation of neural stem cells.⁷²⁻⁷⁷ Glioma stem cells can self-renew in culture with an enhanced proliferation unlike mature glial cells.⁷²⁻⁷⁷ This proliferative property may be, in part, due to the increased activity of the telomerase protein^{88,89} that retards the shortening of the telomeres, and subsequently attenuates cellular senescence or even cell death. Glioma stem cells that form tumourspheres faster have a positive correlation with a more aggressive or higher gradation of gliomas.^{73,75,82}

Glioma stem cells can also differentiate into multi-lineage cells under in-vitro cell culture conditions and

upon in-vivo growth.⁷¹⁻⁷⁵ However, with the heterogeneous population of multi-lineage cells, there is a bias towards the cells that express the astrocytic marker GFAP, and less often cells that express neuronal markers and oligodendrocyte markers. Unlike normal astrocytes, cells differentiated from glioma stem cells express both astrocytic and neuronal markers.⁹⁰ Glioma stem cells also have abnormal karyotypes that are not clonal after subsequent in-vitro passaging in culture.^{74,75,91}

Transformation properties

Glioma stem cells unlike neural stem cells exhibit anchorage independent growth under in-vitro growth conditions. Furthermore, glioma stem cells unlike neural stem cells can grow into tumours upon transplantation into immuno-deficient mice. Most remarkable, the induced tumours recapitulate the vast majority of pathological features of the parental tumour, and even retain tumour-forming potentiation on serial transplantations.^{73,75,82} Within the tumour mass, glioma stem cells have a bias towards forming perivascular niches⁹², possibly as a consequence that this micro-environment rapidly replenishes the pertinent nutrients needed to fulfill their high proliferation and differentiation indices. Secondly, glioma stem cells are highly invasive within the nervous system both locally and distal, compared to neural stem cells. In this manner, they migrate extensively along white matter tracts to distant sites in the host brain, with a

TABLE 2: Similarities and differences between a neural stem cell and glioma stem cell

	Neural Stem Cell	Glioma Stem Cell
Sphere formation	Neurosphere	Tumorsphere
Multi-potency	Yes	Yes
Karyotype	Normal	Abnormal
Self-renewal & proliferation	Yes	Yes (enhanced)
Clonality	High	Low
Tumourigenic	No	Yes
Invasiveness	Low	High

tendency to destroy normal neural stem cells, a phenomenon very similar to the clinical behavior of high grade gliomas in patients.^{2,93}

Pitfalls of glioma stem cell pre-clinical models

The recent emergence in the use of glioma stem cells have contributed to a significant advancement in the development of novel pre-clinical models that have vast recapitulations of the pathological features of the human gliomas.^{73,75,82} However, the major pitfall of glioma stem cell models is the problem of de-novo molecular and pathological signatures that emerge during subsequent progressive passages during in-vitro culturing.⁹¹ This phenomenon is most likely a consequence of their intrinsic genetic instability that is progressively apparent upon in-vitro culturing.⁹¹ Moreover, there is a current notion that the glioma stem cells harvested from the tumour mass when cultured can become inherently different. These current observations underscore the importance of perhaps working with fresh glioma surgical samples and early passaged cells (usually <5 passages), to surpass these pitfalls. For instance, the tumours that arise after transplantation of established cancer cell lines⁹⁴ lose their invasive properties with a tendency of not migrating along the white matter tract, compared to glioma stem cells harvested from fresh glioma surgical specimens.⁹⁰ Secondly, due to inherent clonality problems, glioma stem cell models have variable and spurious therapeutic responses in the context of chemo and radio sensitivity studies.⁸⁰⁻⁸³

Applications of stem cell based pre-clinical models to understanding treatment responses among astrocytoma patients

Of all the stem-cell based models, glioma stem cells are the only one used so far in therapeutic response studies. Nonetheless, described below are recent findings on how current radio- and chemo- therapies affect the glioma stem cell populations within the tumour mass.

Responses to radiation therapy

Radiation therapy has been the principal means to impinge improved survival benefits in the treatments of post-operative patients, especially for those diagnosed with glioblastoma multiforme.⁹⁵ However, recent pre-clinical studies on the glioma stem cell population have demonstrated that these cells are overwhelmingly radio-resistant to clinically relevant doses of radiation⁸⁰, and may therefore be responsible for tumour re-growth and increased aggressiveness after standard treatment measures succumbing to an eventual poor prognosis among treated patients. Specifically, radio-treatments of cells in culture exhibit that the non-glioma stem cell populations of the tumour mass are conducive to apoptosis induced cell death, in contrary to the glioma stem cell populations that remained resilient towards such treatment. In this manner, treated glioma stem cells retained their multi-lineage differentiation property, and most importantly, remained immensely tumorigenic. What is known, thus far, with respect to the molecular mechanisms is that radio-resistance in glioma stem cells is promoted via the constitutive activation of cell cycle regulatory proteins and DNA repair pathway proteins including RAD17, CHK1, CHK2 and ATM.⁸⁰ The importance of these molecular findings is yet to be investigated with relevance towards novel therapeutic targeting strategies.

Responses to chemotherapy drugs

Overall, adjuvant chemotherapy has not been demonstrated to be of major clinical benefit among patients. An exception is the recent report of combined and adjuvant administration of temozolomide, which demonstrated an increase of 14% positive response in the number of glioblastoma multiforme patients who also had a median increase of 2.5 months in prolonged survival, compared to patients treated only with external radiation therapy.^{4,96} In general, most patients develop recurrence or progression after standard treatment measures. Glioma stem cells have elegant multi-

drug resistance that is mediated via several mechanisms.⁸¹ Genes implicated in such multi-drug resistance include BCRP1. BCRP1 is a member of the ATP binding cassette (ABC) transporters comprising of 48 members.⁹⁷ These transporters traffic molecules such as lipids and xenobiotic compounds across the cell membrane in an ATP dependent mechanism⁹⁷. Another gene is MGMT, which is required to repair O⁶-methylguanine DNA induced damage by drugs such as temozolomide. If left un-repaired, this modified base can mis-pair with deoxy-thymidine during DNA replication, succumbing to acquired mutations and increased genetic instability that may potentially induce cell death.⁹⁸ Anti-apoptosis pathway genes such as BCL2, FLIP, BCL-XL and IAPs (XIAP, cIAP1, cIAP2, NAIP and SURVIVIN) also confer chemo-resistance in glioma stem cells upon treatment with a plethora of chemo-therapy drugs. These genes also have an association with chemo-resistance in other cancers including acute myeloid leukemia, non-small cell cancer and breast cancer.⁸¹ Studies are yet to be explored which specifically address the use of combined targeted therapies, including novel drugs, that can inhibit these multi-drug resistance pathways. However, one recent and significant progress has been achieved by the identification of at least 160 new drugs that seem to effectively eradicate the glioma stem cell populations.⁹⁹ The mechanisms of how these novel drugs mediate such pharmacological responses are yet to be elucidated.

Concluding remarks

Cancer associated deaths are an emerging epidemic in North America. Amongst all brain tumour cases, patients diagnosed with astrocytomas are the most common. Unfortunately despite current treatments, the majority of patients demonstrate little or no prospects for a successful prognosis. The overall impact on the healthcare system for treatments is progressively becoming more expensive. Furthermore, the psychosocial constraints on the families are devastat-

ing. These treatment failures are predominantly due to the current lack of robust pre-clinical models that recapitulate the pathological, molecular and treatment characteristics of the human cancers. The recent discoveries of stem cells or immature/early-differentiated cell types as being more reminiscent in the origin of astrocytomas; and the development of stem-cell based pre-clinical models, are pre-requisites towards a fruitful venue in our quest to understand the biology of astrocytomas, and most importantly with applied prospects for more effective translational therapeutic studies. These models and applied therapeutic intervention studies will eventually assist towards a bench to bedside transition in our collective effort towards the eradication of this deadly disease among those affected.

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