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Review

Using bioluminescence imaging in glioma research

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ABSTRACT

Glioblastoma multiforme (GBM) is the most common malignant brain tumour and has the worst prognosis. Over the last decade, the use of bioluminescence imaging technology has rapidly become widespread to further understand the mechanisms that drive GBM development and progression. Preclinical evaluation and optimisation of therapeutic efficacy in GBM research has also utilised this simple non-invasive technology. Here we summarise recent advances made in glioma biology and therapeutic intervention using bioluminescence imaging. This review also describes the current knowledge regarding the use of luciferase-based reporters in examining the role of specific cancer signalling cascades that promote glioma progression.

1. Introduction

Glioblastoma multiforme (GBM), also known as World Health Organization grade IV astrocytoma, is the most common malignant primary tumour of the central nervous system [1,2]. Present therapies are only effective in short-term control of the tumour and recurrence is inevitable despite the use of conventional treatment [3–6]. Translational efforts to discover critical promoters of glioma progression and to evaluate novel agents in glioma intervention have been assisted by the use of bioluminescence imaging (BLI), which allows for non-invasive, longitudinal evaluation of pre-clinical therapeutics [7]. BLI is based upon the detection and quantification of light emission generated from the conversion of chemical energy into photon energy [8,9]. This occurs via the oxidisation of a substrate (primarily D-luciferin) by the enzyme luciferase within live cells and tissue [8,9]. Several luciferase genes cloned from varying organisms have been utilised, including firefly luciferase (Fig. 1), renilla luciferase, gaussia luciferase and most recently vargula hilgendorfii luciferase [10], with firefly luciferase most routinely used [9].

BLI is now commonly employed to examine potential therapeutics in a number of brain tumour types including medulloblastoma [11], oligodendroglioma [12], atypical teratoid rhabdoid tumours [13], diffuse intrinsic pontine glioma [14], and metastasis to the brain from other tissue outside the brain [15–17]. In addition, BLI technology has also been applied for the monitoring of a mouse model of neurofibromatosis [18], a syndrome in which predisposed individuals have a greater incidence of tumours of the nervous system. Here we will focus specifically of the role that BLI has played in assisting the evaluation and optimisation of both standard and novel therapeutics in the high-grade glioma setting using both cell culture systems and live animal imaging. We will also discuss the current use of BLI in furthering our understanding of the molecular mechanisms that drive glioma progression.

2. Translational applications using BLI

Many groups have engineered human glioma cell lines and primary cultures to stably express a luciferase gene through either transfection of a DNA construct or infection of lentiviral vectors. These cells are suitably equipped as diagnostic tools to monitor large scale drug screening and for drug evaluation and optimisation in a biological setting. Many of these tests have been performed on GBM cell lines. We will review these studies in the following sections.

2.1. Evaluating and optimising chemotherapy for the treatment of GBM

A pivotal study successfully using BLI as a surrogate marker for orthotopic brain tumour growth and chemotherapeutic efficacy was first published in 2000 by Rehemtulla and colleagues [19]. They showed that the intracranially grown rat 9L gliosarcoma cells stably transfected with the luciferase gene could be non-invasively quantified by detection of bioluminescent photon emissions which

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were proportional to tumour size. In addition, quantitation of the inhibitory effects of the chemotherapeutic 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) was achieved again by BLI in rats pre and post-administration comparing untreated control *versus* BNCU treated rats [19].

As surgical resection followed by post-operative temozolomide and radiotherapy is currently the standard of care for GBM patients [20–22] it is not surprising that a number of groups have sought to examine and enhance the effects of temozolomide in translational animal models using BLI. Kemper and colleagues tested the efficacy of temozolomide administered as daily oral doses of 100 mg/kg for 5 days. Response to temozolomide was determined by the level of bioluminescence photons emitted, and images from control treated mice *versus* temozolomide treated mice were compared. Temozolomide could significantly reduce relative bioluminescence intensity in human glioblastoma cell line U87MG stably incorporated with a firefly luciferase gene, but had no effect on the rat glioblastoma cell line RG-2 [23]. Unlike the study by Kemper and colleagues, a subsequent study by another group correlated the ability of temozolomide to mediate change in bioluminescence intensity in intracranially grown GBM14 tumours with the overall survival of the mice, thereby justifying the use of BLI as a surrogate marker for efficacy of therapeutic agents in intracranial mouse models [24]. Furthermore, their report revealed that low-dose protracted temozolomide treatment (50 mg/kg/daily for 5 consecutive days) reduced relative photon units and enhanced the survival of mice significantly longer than mice receiving single high-dose temozolomide (120 mg/kg) [24]. In addition, another study utilised BLI to evaluate temozolomide efficacy on glioma xenografts injected in the brainstem of athymic rats [14]. In an attempt to establish a pre-clinical model for therapeutics targeting brainstem gliomas, Hashizume and colleagues injected two glioblastoma cell lines (U87MG and GS2) into the pontine tegmentum of athymic rats using a guide-screw system. Established xenografts treated with temozolomide (50 mg/kg/daily orally for 5 consecutive days) displayed significantly reduced relative bioluminescence activity compared to xenografts from control treated rats. In addition, this temozolomide treatment significantly enhanced animal survival compared to the control treated rats [14].

Temozolomide exerts its cytotoxic activity by methylating several specific DNA sites including the O^6 position of the nucleotide guanine, resulting in cell death [25]. The DNA repair protein O^6 methylguanine-DNA methyltransferase (MGMT) directly inhibits the cytotoxic effect of temozolomide by removing the methyl group from the O^6 position of guanine in DNA [26,27]. Subsequently, over-expression of MGMT in GBM has been identified and this also correlates with resistance to temozolomide. Conversely, reduced MGMT expression through methylation of the MGMT promoter, results in enhanced sensitivity to temozolomide treatment [28]. Thus reducing MGMT expression has been proposed as a possible therapeutic intervention to re-sensitise GBM cells to the cytotoxic effects of temozolomide. Indeed, a recent report showed that a lentiviral vector expressing luciferase and shRNA for MGMT could transduce LN18 subcutaneous xenografts and enhanced the growth inhibitory effect of temozolomide [29].

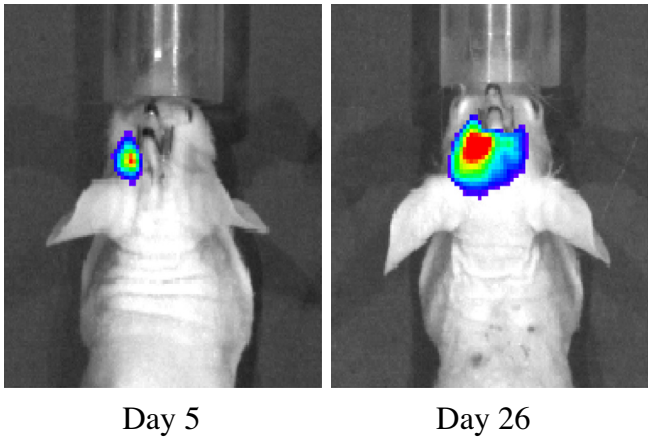

Fig. 1. Bioluminescent imaging of a mouse brain bearing human tumour cells. U87MG cells (1×10^6) stably transfected with the CMV driven firefly luciferase construct (*pGL4-CMV-Fluc*) were injected intracranially into BALB/$c^{nu-/nu-}$ mice. Five and 26 days later mice were imaged for luciferase activity using an IVIS Lumina (PerkinElmer, Waltham, MA, USA) bioluminescence system. Visualisation of luciferase activity was achieved using the IVIS Lumina by injecting mice intraperitoneally with 150 mg/kg of the luciferase substrate, D-luciferin.

Others have also utilised BLI to assess the efficacy of temozolomide in glioma xenograft mouse models in combination with another agents. The combination of temozolomide and an adenovirus targeting the Y-box binding protein 1 led to enhanced reduction in growth of subcutaneous U87MG tumours compared to a single agent treatment and control treatment in mice as determined by reduced tumour volume and BLI [30]. Likewise, an inhibitor to p53 and a dual inhibitor targeting PI3K/mammalian target of rapamycin (mTOR) signalling reduced the BLI signal intensity and enhanced the cytotoxic and inhibitory effects of temozolomide in intracranial GBM mouse models [31,32]. Furthermore, the combined efficacy of rolipram, an inhibitor to the cyclin AMP phosphodiesterase-4, together with temozolomide and radiation, was assessed on luciferase transfected U87MG intracranial xenografts [11]. Rolipram in combination with temozolomide and radiotherapy enhanced the overall survival of mice bearing U87MG intracranial tumours and decreased BLI signal intensity compared to the control groups [11]. Finally, the use of genetically modified human mesenchymal stromal cells (hMSC) as potential carriers of cytotoxic agents such as chemotherapeutics that specifically localise at the tumour site has been evaluated in intracranial models of GBM. Most recently, a study showed that treatment with tumour necrosis factor–related apoptosis-inducing ligand (TRAIL) secreting hMSC combined with temozolomide could significantly enhance survival of mice bearing intracranial glioma tumours and was coupled with reduced BLI signal intensity in these treated tumours [33].

Others have evaluated the efficacy of other chemotherapeutics in glioma orthotopic models utilising BLI. The study by Chen and colleagues evaluated the anti-tumour effects of single delivery irinotecan in nanoliposomal particles by convection-enhanced delivery (CED) *versus* multiple systemic intravascular administrations [34]. They observed that nanoliposomal irinotecan intratumourally delivered by CED reduced the BLI signal intensity in GBM43 and SF7796 GBM xenografts to a significantly greater degree than vascular administration. In addition CED enhanced mouse survival when compared to vascular administration of the nanoliposomal irinotecan [34]. Intravenously administered irinotecan in combination with the anti-vascular endothelial growth factor (anti-VEGF) monoclonal antibody, bevacizumab has also been evaluated in intracranial models, producing enhanced anti-tumour effects as determined by BLI [35]. These authors also determined that the cytotoxic drug NK012 could reduce the BLI signal intensity of luciferase-labelled U87MG tumours and prolonged survival times of mice bearing these NK012 treated tumours [35].

Likewise, a series of recent papers has utilised BLI to examine the efficacy of liposomal doxorubicin and CED in intracranially grown glioma cell lines. Liposomal doxorubicin significantly reduced the orthotopic growth of luciferase-labelled 8401 and U87MG glioma cells, while doxorubicin complexed with nano-diamonds and administered by CED significantly reduced growth of luciferase-labelled rodent glioma cells as determined by reduced bioluminescence intensity [36–38].