Radiation-Induced Changes in the Glioma Microenvironment

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Malignant gliomas are the most common primary brain tumour of which glioblastoma (GBM) is the most prevalent and biologically aggressive (incidence of five to eight cases per 100,000 population/year in the UK). The propensity of gliomas to infiltrate the normal brain means that complete surgical excision is impossible without causing significant neurological deficit. Following surgery, patients typically undergo a course of radiotherapy and when indicated (i.e. good performance status), a combination of chemo/radiotherapy ("Stupp" protocol)[1]. However, in spite of this multi-modal therapeutic approach, median survival is typically no more than 12-14 months.

While radiation forms the basis of post-surgical treatment and confers undoubted survival benefits, tumour relapse close to the primary site is inevitable [2]. Considerable research into the mechanisms of this radiation resistance has focused on DNA damage repair mechanisms [3] and the role of the glioma "stem-like" population [4,5]. More recent evidence is emerging that radiation treatment in itself may promote glioma survival and invasion via interactions with the host microenvironment [6-8]. These pro-tumourigenic effects can be mediated either directly by the tumour cells itself and/or via the normal cells within the brain stroma. These observations stem from a range of experimental models and highlight the importance of considering the potential adverse effects of radiation in patients and the need to counter them.

Direct effects of radiation on glioma cells

Although malignant gliomas have an inherent invasiveness, evidence is emerging that irradiation of GBM cells *in vitro* can result in an induction of various factors involved in glioma invasion [6]. These include enhanced expression of tumour cell surface adhesion molecules (e.g. $\alpha v\beta 3$-integrin) and proteases which promote attachment and degradation of the surrounding extracellular stroma to facilitate glioma cell migration and invasion (e.g. metalloproteases) [9-11]. Preliminary work by our group indicates that the cysteine protease Cathepsin S, which we have previously shown to be involved in glioma invasion [12-14], may also be up-regulated in glioma stem-like cells by irradiation (Figure 1A). In an ongoing collaborative study with the Centre for Image-guided Neurosurgery at the University of Pittsburgh, we have observed high levels of both tumour and microglial Cathepsin S expression in tumour specimens from multiply irradiated GBM patients (Figure 1B).

The role of irradiated brain stroma on glioma biology

Although sub-lethal radiation delivered directly to glioma cells may result in an increased invasive potential, there is evidence these irradiated cells may also release signals into the tumour microenvironment, modifying the phenotype of both non-irradiated glioma and normal brain stromal cells, known as the "bystander effect" [15]. A range of signaling molecules and pathways are involved and activated in this process, including cytokines (e.g. chemokine, CXCL8), reactive oxygen/nitrogen species, prostaglandins, and mitogen-activated protein kinase pathways (MAPKs) [15,16]. These signaling factors may recruit host inflammatory cells, in particular microglia/macrophages, into the glioma microenvironment to execute many tumourigenic processes such as invasion, angiogenesis and promoting glioma cell survival [17,18]. Using a syngeneic mouse glioma model, Chiang and colleagues demonstrated that irradiation promoted an M2 macrophage phenotype under hypoxic conditions – a characteristic feature of the GBM microenvironment [19]. It is clear that if we are to define appropriate scheduling of radiotherapy alongside molecular targeting, a greater understanding of the microenvironment is crucial.

The need for quantification of radiation effects in GBM patients

A range of *in vitro* and *in vivo* experimental models are useful to explore mechanisms and the effects of radiation-induced tumourigenesis, but they may not accurately reflect the complex glioma-brain microenvironment found in humans. Another potential confounding factor in experimental models is the blood-brain barrier and the influence this may have on tumour biology, therapeutic delivery and response. It is therefore important to correlate these experimental findings with clinical studies. New approaches involving the use of targeted irradiation protocols with small animal irradiation platforms [20] and orthotopic models offer tremendous potential and endeavour to more precisely replicate clinical protocols in preclinical models [21].

Despite its established role in glioma treatment, there are relatively few studies which have quantified the effects (both beneficial and harmful) of radiotherapy in malignant gliomas beyond the radiation necrosis/pseudo-progression/true progression debate [22]. Furthermore, with the increasing knowledge of the molecular heterogeneity of GBM, there have been few studies to determine which groups of patients might respond best to radiotherapy [23,24]. A transcriptomic array study by Ducray and colleagues indicated that differential expression of microenvironment genes was associated with responses to radiotherapy [25]. The expression of hypoxia-related genes was associated with short-term progression-free survival (< 5 months), whereas the expression of immune genes was associated with prolonged progression-free survival (> 10 months). Indeed, the concept for stratifying elderly GBM patients into either upfront

chemotherapy or radiotherapy is gathering momentum based on the tumour molecular profile (e.g. MGMT promoter methylation status) in light of recent randomised clinical trials [26].

With the evolution of radiotherapy technologies including IMRT, stereotactic radiotherapy, intra-tumoural brachytherapy (e.g. GliaSite), and advances in brain imaging techniques (MR perfusion, spectroscopy, PET imaging, volumetric analysis of contrast- and non-contrast-enhancing tumour volumes), there is the opportunity to predict which patients will respond best to radiation treatment and how to best monitor their treatment response [27-30]. Here at the Centre for Cancer Research and Cell Biology in Queen's University Belfast together with the Belfast NHS Trust and local pharmaceutical industry, a multidisciplinary team of neuro-oncologists, radiation oncologists, molecular pathologists, and radiation, cancer and omics systems biologists are working to deliver a translational pipeline of new approaches in glioblastoma therapy focussed on radiation-mediated microenvironmental changes. The ultimate goal is to develop stratified therapeutic approaches, based on a greater understanding of the key molecular processes that drive resistance and invasion, to improve the quality of life and survival in GBM patients. ■

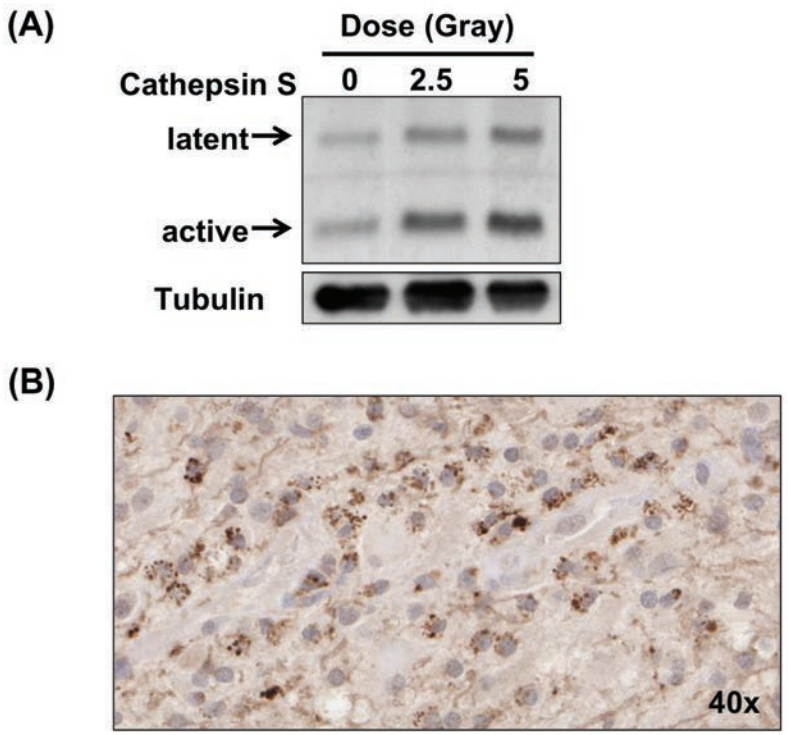

Figure 1.
(A) Radiation induces increased expression of Cathepsin S in glioma stem-like cell cultures *in vitro*. Both the latent and active forms of Cathepsin S are up-regulated in a dose-responsive manner following X-ray irradiation.
(B) Immunohistochemical staining of Cathepsin S in patient biopsy sections following multiple radiation treatments. Particulate staining is indicative of its lysosomal localisation in both tumour and microglial cells.

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