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Review

Targeting hypoxia in the treatment of small cell lung cancer

J.L. Bryant[a,b], S.L. Meredith[b], K.J. Williams[b], A. White[a,b,*]

[a] Faculty of Life Sciences, Manchester Academic Health Sciences Centre, University of Manchester, AV Hill Building, Manchester M13 9PT, UK
[b] Faculty of Medical and Human Sciences, Manchester Academic Health Sciences Centre, University of Manchester, AV Hill Building, Manchester M13 9PT, UK

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ABSTRACT

Small cell lung cancer (SCLC) is an extremely aggressive disease for which minimal therapeutic improvements have been made over the last few decades. Patients still rely on non-targeted, chemotherapeutic drugs complemented by irradiation. Although initial response is very good, the majority of SCLC patients invariably relapse with therapy-resistant tumours. Despite the link between pathologically low oxygen levels and therapy resistant tumours, hypoxia has gained little attention in the development of novel therapies for SCLC. In contrast, the advantages of targeting hypoxic cells in many other cancer types have been studied extensively. This review describes the reasons for targeting hypoxia in SCLC and outlines strategies undertaken to enhance hypoxic tumour cell death, including the use of bioreductive prodrugs, the targeting of HIF-1α and the induction of cell death through acidosis. Therapy directed towards hypoxic tumour regions has the potential to greatly enhance the response of SCLC tumours to current treatment regimens and represents an area of research in need of greater attention. Such research could lead to the much sought after development of targeted drugs against SCLC tumours.

1. Introduction

Lung cancer is the leading cause of cancer deaths worldwide, with almost 1.6 million deaths attributed to the disease in 2012 alone. Median survival lies at less than 12 months, largely due to early metastatic dissemination and resistance to therapy [1].

Small cell lung cancer (SCLC) accounts for 10–15% of all lung cancer cases and represents the most aggressive subset of lung cancer. Treatment of SCLC has changed minimally over the last few decades. Patients continue to receive non-targeted, chemotherapy regimens consisting of etoposide plus a platinum agent, often combined with irradiation. Although SCLC patients respond well to this first-line treatment, relapse is virtually inevitable and resultant tumours are resistant to further therapy [2].

Multiple studies have highlighted the presence of hypoxia in SCLC tumours [3–5]; however, there are no hypoxia-targeted therapies currently in use to our knowledge. This review aims to provide an understanding of the role that hypoxia plays in tumour pathobiology in order to provide evidence as to why therapy targeted at hypoxic regions would be highly beneficial in the treatment of SCLC.

2. Hypoxia in cancer

Hypoxia is an important microenvironmental pressure present in the majority of solid tumours. Due to rapid proliferation of tumours, cells outgrow their surrounding vasculature and diminish available oxygen supplies, resulting in a drop from the normal 2–9% oxygen to hypoxic levels below 2% [6]. This induces a switch to glycolysis, which is thought to present a more efficient method of energy production in cells taking up higher levels of glucose [7].

Although not all tumour cells are under hypoxic conditions, cancer cells have a tendency to convert from aerobic respiration to glycolysis, even in the presence of oxygen. This phenomenon, termed the Warburg effect, results in many adaptations associated with hypoxic cells, which lead to enhancement of aggressive characteristics and therapy resistance in tumour cells [8,9]. This further supports the use of hypoxia-targeted drugs when treating tumours.

2.1. Hypoxia in small cell lung cancer

Histological examination of SCLC biopsies suggests that at least half of all newly diagnosed SCLC patients have tumours displaying hypoxic regions [10]; a figure likely to be much higher when

* Corresponding author at: Faculty of Life Sciences, University of Manchester, 3.016 AV Hill Building, Manchester M13 9PT, UK. Tel.: +44 161 275 5178; fax: +44 161 275 5958.
E-mail address: awhite@manchester.ac.uk (A. White).

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taking into account the small size of biopsies and their inability to represent full intra-tumour heterogeneity.

SCLC patients respond extremely well to first-line chemotherapy, but almost invariably relapse with therapy-resistant disease. Despite the link between hypoxia and therapy resistance, hypoxia-targeted therapy for SCLC remains relatively under-explored, whereas a wealth of data exists supporting the role of such therapy in many other cancers.

2.2. Hypoxia induces cellular adaptations in tumour cells

Multiple cellular changes arise as a result of hypoxia that are regarded as hallmarks of cancer [11]. These include enhanced proliferation [12], increased metastasis [4], resistance to therapy [13] and de-differentiation [14].

Tumour cells exposed to low oxygen concentrations up-regulate hypoxia-inducible factor-1α (HIF-1α), which in turn controls many factors. There are, however, a number of adaptations that occur through HIF-independent pathways, for example the down-regulation of pro-apoptotic proteins, Bid and Bax [15] and translational enhancement of vascular endothelial growth factor (VEGF)-C [16].

The activation of HIF-1α signalling is widely considered the most important adaptation for survival under low oxygen conditions. Among the hundreds of targets of HIF-1α, two that have been studied extensively are VEGF and carbonic anhydrases (CAs) [17,18].

VEGF is important for the induction of angiogenesis in an attempt to restore oxygen and nutrient flow to poorly vascularised tumour regions [19]. CA IX is a tumour-specific enzyme involved in pH maintenance within hypoxic regions [18]. Both molecules are associated with poor survival in lung cancer patients and have been studied as potential therapeutic targets [20,21].

2.3. Hypoxia is associated with resistance to therapy

Resistance to therapy in the presence of hypoxia was noted as early as the 1920s [22], with the first clinical implications observed in lung cancer tumours exhibiting resistance to ionising radiation [23]. It has since been shown that in oxygenated cells, radiotherapy produces DNA-damaging free radicals. Under hypoxic conditions, these free radicals are rapidly reduced, thereby avoiding DNA damage [6]. Since then, the ability of hypoxic cells to evade radiotherapy and chemotherapy has been studied extensively and observed in a large number of patients [15,24–29]

There are various possible mechanisms contributing towards chemotherapy resistance in hypoxic cells [30]. An obvious problem is that hypoxic regions are further from blood vessels, making it difficult for drugs to diffuse and reach these cells [28]. Some drugs may also require oxygen in order to exhibit their toxic effects, as evidenced by bleomycin used to treat a range of cancers [31]. Drug-induced apoptosis can often be avoided in hypoxic cells through down-regulation of pro-apoptotic proteins, such as Bid and Bax [15,25]. Although the effects of hypoxia on the response to chemotherapy have been studied in great depth, little is known about the effect that chemotherapy has on hypoxia [30].

Etoposide, cisplatin and doxorubicin are three commonly used chemotherapeutic agents in various cancers, including SCLC. Treating cancer cell lines with these agents in the absence of oxygen resulted in a marked increase in resistance [24,25]. Sensitivity to cisplatin and doxorubicin can be restored in non-small cell lung cancer (NSCLC) cells when ablating HIF-1α activity and subsequent hypoxic responses [29].

Hypoxia is common within tumours and cells occupying these regions possess an enhanced ability to evade many types of therapy. This emphasises the necessity to target hypoxic cells when developing novel approaches for cancer therapy.

3. Hypoxia-targeted therapy for SCLC

Attempts to target hypoxia in SCLC cells have been limited despite the prevalence of hypoxia within tumours. Lung cancer patients experience breathing difficulties as a result of obstruction caused by tumours, tar from smoking or chronic obstructive pulmonary disease (COPD), leading to difficulties in blood and tissue oxygenation. Furthermore, chemotherapy induces anaemia in many patients, reducing the ability to transport oxygen in the blood [32]. These additional factors stress the importance of targeting tumour cells in hypoxic conditions.

Current therapeutic efforts focus on targeting hypoxic cells through the use of bioreductive prodrugs and the inhibition of molecular targets up-regulated in these cells [33].

3.1. Use of bioreductive prodrugs and radiosensitizers to target hypoxic cells

Tumour cell death has been enhanced by the use of bioreductive prodrugs since the 1960s [34]. These prodrugs are activated under the highly reductive conditions found within hypoxic environments and, in the majority of cases, interfere with DNA replication, leading to cell death [35]. The capacity for bioreductive prodrugs to enhance the killing effects of both irradiation and chemotherapy give them great potential in the treatment of small cell lung cancer.

Tirapazamine remains the most advanced clinical bioreductive drug; however, initial phase III clinical trials have failed to show great benefit for NSCLC and head and neck cancer [36]. The effects of tirapazamine are limited by rapid metabolism when leaving the vasculature and subsequent inability to diffuse into hypoxic regions. Currently, no clinically approved bioreductive prodrugs exist, but many are under investigation and entering clinical trials [37].

In addition to bioreductive prodrugs, a number of radiosensitizing agents have been developed that work by enhancing the sensitivity of hypoxic regions to radiation therapy, often through mimicking the effects of oxygen [26]. Oxygen itself can also be administered as a radiosensitizing agent, as discussed later.

3.2. Targeting HIF to enhance tumour cell death

HIF comprises two subunits; the α subunit expressed transiently in the cytoplasm and the β subunit present constitutively in the nucleus. The HIF-α subunit exists in three isoforms; the most researched being HIF-1α. Under normoxic conditions, HIF-1α is targeted for proteasomal degradation by prolyl hydroxylase domain (PHD) proteins, whereas under hypoxia, HIF-1α is able to enter the nucleus and bind to HIF-1β to coordinate gene expression of more than 200 target genes (Fig. 1) [38]. Expression of HIF-1α has been linked with poor survival in multiple cancers, which rely on HIF signalling to induce cellular adaptations that enable them to survive under hypoxic conditions [4,25,39]. Both HIF-1α and HIF-2α expression correlates with poor survival in SCLC patients and therefore make attractive molecular targets [4,40].

Inducing HIF-1α degradation in NSCLC using rapamycin or curcumin increases apoptosis and sensitisation to cisplatin, respectively [41,42]. Similarly, in mice bearing Lewis lung carcinomas, survival was prolonged when HIF-1α and HIF-2α were knocked down using siRNA [43]. Although a limited number of studies have been carried out in SCLC cells, early xenograft experiments involving HIF-1α knock down have proven extremely effective [4]. SCLC cells *in vitro*, however, have shown contradictory results whereby inhibition of HIF-1α has no effect on survival [3]. This may highlight