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ANTI TUMOUR TREATMENT

The cellular adaptations to hypoxia as novel therapeutic targets in childhood cancer

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Summary Exposure of tumour cells to reduced levels of oxygen (hypoxia) is a common finding in adult tumours. Hypoxia induces a myriad of adaptive changes within tumour cells which result in increased anaerobic glycolysis, new blood vessel formation, genetic instability and a decreased responsiveness to both radio and chemotherapy. Hypoxia correlates with disease stage and outcome in adult epithelial tumours and increasingly it is becoming apparent that hypoxia is also important in paediatric tumours. Despite its adverse effects upon tumour response to treatment hypoxia offers several avenues for new drug development. Bioreductive agents already exist, which are preferentially activated in areas of hypoxia, and thus have less toxicity for normal tissue. Additionally the adaptive cellular response to hypoxia offers several novel targets, including vascular endothelial growth factor (VEGF), carbonic anhydrase, and the central regulator of the cellular response to hypoxia, hypoxia inducible factor-1 (HIF-1). Novel agents have emerged against all of these targets and are at various stages of clinical and pre-clinical development. Hypoxia offers an exciting opportunity for new drug development that can include paediatric tumours at an early stage.

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Introduction

Despite dramatic improvements in treatment for childhood cancer, such that 75% of children will now be cured of their disease, it remains the commonest cause of death in children between 1 and 14 years in the UK. There remain many tumour types in which survival is poor despite aggressive treatment protocols incorporating intensive chemotherapy, radiotherapy and surgery. Children with stage 4 neuroblastoma, alveolar rhabdomyosarcoma, many types of brain tumour and metastatic Ewing's sarcoma and osteosarcoma still have 5-year survival rates less than 60%.[1] There is only limited scope for improvement with conventional chemotherapy and thus there is an urgent need for novel agents for these patients.

Tumour-related hypoxia is now widely recognised as a cause of treatment failure and poor outcome for a wide variety of adult malignancies, especially in relation to the treatment of carcinomas. Tumour hypoxia is known to increase resistance to both radiotherapy[2] and many cytotoxic agents.[3] Hypoxia also has profound effects on the biology of tumours and their surrounding stromal cells, with increased invasion, angiogenesis and metastases being characteristic of hypoxic malignancies.[4] The cellular adaptations of cancer cells to hypoxia affect transcriptional regulation,[5] increase glucose uptake capacity and glycolysis,[6] stimulate cell motility and invasion[7] and regulate apoptosis.[8] These hypoxia-induced changes confer an advantage to cancer cells in terms of proliferation and metastatic spread and reduce the sensitivity of the cancer cells to treatment.

Targeting hypoxia is an exciting prospect for the therapy of children's cancers, and the aim of this review is to explore the effect of hypoxia in tumours, particularly in terms of drug resistance, and highlight the potential importance of hypoxia as a target for therapy in paediatric malignancies.

Tumour hypoxia

Hypoxia, a reduction in the level of tissue oxygenation to 7 mmHg or less, is a feature of nearly all solid cancers.[9] Direct measurement of tumour oxygenation by 'eppendorf' micro-electrode shows solid tumours to be significantly more hypoxic than surrounding normal tissue but also that a large range of oxygenation levels exists within tumour tissue.[4,10] Measuring oxygen levels using the eppendorf electrode remains the 'gold standard' but the technique is invasive and not without its problems including inability to differentiate between tumour and stromal cells, areas of necrosis, acute or chronic hypoxia and operator dependant variations. Surrogate markers of hypoxia have thus been widely sought and correlation between direct pO_2 measurements and markers such as hypoxia inducible factor-1alpha (HIF-1a)[11], vascular endothelial growth factor (VEGF),[12] the facilitive glucose transporter Glut-1[13] and carbonic anhydrase IX (CA IX)[14] have been found in some studies but not in others.[15,16] These conflicting results may be due to inherent difficulties in the techniques themselves or to the influence of oxygen independent factors, such as growth factors and oncogenes, on these signalling pathways. These non-hypoxic influences raise caution over the use of such indices as surrogate markers of hypoxia. However their value clinically can be in little doubt; increased expression of HIF-1a, VEGF, Glut-1 and CA IX have all related to an adverse outcome for a wide variety of adult cancers.[17] The presence of these hypoxia related markers have been demonstrated in several paediatric tumour types, ranging from childhood ALL[18] to neuroblastoma,[19] although studies of the biology of hypoxia in childhood cancers remain limited. Non-invasive imaging techniques for evaluating hypoxia are emerging that may be able to guide us clinically, enabling stratification of patients based on tumour hypoxia (for review see Tatum et al.[4]) and also clarifying the relationship between oxygen levels and tumour biology.

Cellular adaptation to hypoxia

Many fundamental biochemical pathways — DNA synthesis, transcription, translation, gene regulation and energy production — first developed under conditions of anoxia. Indeed, there are a number of biological processes, such as anaerobic glycolysis, that function best in hypoxia[20] and it is these pathways that are up-regulated in the hypoxic cancer cell. The complex interactions between the various biological pathways enabling both malignant and non-malignant cells to adapt to a hypoxic environment are beginning to be unravelled. Key molecules have emerged as vital to this adaptive process. HIF-1 is the key transcription factor that up-regulates the expression of genes which allow both normal and malignant cells to adapt to hypoxic conditions. HIF-1 exists as a heterodimer of the hypoxic response factor HIF-1a and HIF-1b (also called aryl hydrocarbon receptor nuclear translocator, ARNT).[21] Whilst HIF-1b is constitutively expressed, HIF-1a levels are normally kept low through proteasomal degradation. The ubiquitination and targeting for degradation of HIF-1a is mediated through its binding to the protein product of the Von Hippel—Lindau tumour suppressor gene under conditions of normal oxygen tension.[22—24] Under hypoxic conditions the modification of HIF-1a by oxygen dependant prolyl hydroxylases necessary for this interaction does not occur, meaning that it is able to dimerise with HIF-1b, bind to hypoxia response elements (HREs) in target genes and activate them.[25] HIF-1 is known to regulate the expression of more than 70 target genes,[5] the products of which promote neo-angiogenesis and regulate VEGF production, up-regulate the glycolytic pathway, promote anaerobic glycolysis, and regulate apoptosis[26] (Fig. 1). Its over-expression in human cancers is widespread. In paediatric cancers it is over-expressed in Wilm's tumour[27] and in the bone marrow of ALL patients and neuroblastoma patients with bone metastasis.[18] The HIF-1 pathway can also be activated by other mechanisms including growth factors, activation of oncogenes (HER2neu,[28] H-RAS[29]) or inactivation of tumour suppressor genes (p53, VHL, PTEN)(for review see Bardos and Ashcroft, 2004[30]). The activation of HIF-1 by these mutations in cancer promoting genes illustrates the profound influence HIF-1 and its effectors have on the survival of the cancer cell.

Energy metabolism and anaerobic glycolysis

Continued metabolism of glucose is the mainstay of cellular adaptation to hypoxia. Expression of molecules involved in