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PUSHING THE FRONTIERS OF RADIOBIOLOGY: A SPECIAL FEATURE IN MEMORY OF SIR OLIVER SCOTT AND PROFESSOR JACK FOWLER: COMMENTARY

Targeting tumour hypoxia: shifting focus from oxygen supply to demand

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ABSTRACT

Tumour hypoxia is a well-recognised barrier to anti-cancer therapy and represents one of the best validated targets in oncology. Previous attempts to tackle hypoxia have focussed primarily on increasing tumour oxygen supply; however, clinical studies using this approach have yielded only modest clinical benefit, with often significant toxicity and practical limitations. Therefore, there are currently no anti-hypoxia treatments in widespread clinical use. As an emerging alternative strategy, we discuss the relevance of inhibiting tumour oxygen metabolism to alleviate hypoxia and highlight recently initiated clinical trials using this approach.

The presence of sub-physiological levels of oxygen (hypoxia) is a common feature of many solid tumours. As tumours rapidly grow and proliferate, high oxygen demand surpasses supply due to an invariably dysregulated and "chaotic" tumour microvasculature.[1] Tumour hypoxia is a robust negative clinical biomarker and is considered, by many, as one of the best validated targets in oncology. The detrimental impact of tumour hypoxia on cancer treatment outcomes plays a particularly important role in radiotherapy, as first realised over 60 years ago.[2] Severe (radiobiological) hypoxia leads to a reduction in the fixation of radiation-induced DNA damage and therefore reduced treatment efficacy. As Gray and Scott's seminal work demonstrated, hypoxia renders cancer cells up to three times more resistant to ionising radiation—a phenomenon known as the "oxygen effect".[2] As the importance of overcoming tumour hypoxia became increasingly realised, a number of strategies were developed and tested clinically in combination with radiotherapy. However, such efforts yielded only modest benefits, resulted in significant toxicity, or were often impractical in the clinical setting. Consequently, there are no anti-hypoxia treatments in widespread clinical use today. The development of novel and clinically applicable strategies to alleviate hypoxia is needed as much today as it was over half a century ago.

Early attempts to alleviate hypoxia involved the use of carbogen (5% CO_2 and 95% O_2) or the delivery of oxygen via hyperbaric oxygen chambers. When combined with vasodilating agents, it was hoped that increased oxygen delivery would translate to more oxygen being available to diffuse throughout the tumour. Unfortunately, in spite of promising pre-clinical and early-phase data, such approaches did not translate into significant improvements in randomised clinical studies, while simultaneously raising concerns about practicality[3] and safety.[4]

The use of hypoxia-activated pro-drugs was also explored in an effort to target hypoxic cells directly. Such compounds become preferentially activated and reduced to form cytotoxic species in low oxygen tension. This effect is designed to spare well-oxygenated tissues to thereby yield a larger therapeutic window. The most well-known molecule in this class is tirapazamine, which has been shown to be 300-times more cytotoxic under hypoxia.[5] Unfortunately, studies using tirapazamine as a single agent or combined with chemoradiotherapy did not demonstrate significant efficacy.[6] Other such bioreductive compounds have also been studied including N-oxides, quinones and metal complexes, but similarly demonstrated modest clinical efficacy or unacceptable toxicity. Indeed, it is possible that the use of this class of drugs may be intrinsically limited since

they are required to reach areas of tumours that are, by definition, perfusion-restricted.

The use of pharmacological hypoxic cell radiosensitisers that promote the fixation of free radical damage by acting as oxygen mimetics has also long been of interest in radiation oncology. Well-known examples of such compounds are misonidazole and nimorazole. Trials involving the former were terminated due to toxicity[7] while the latter continues to be investigated (NIMRAD trial).[8] Randomised clinical trials combining nimorazole with radical radiotherapy in head and neck cancer, namely DAHANCA 5, demonstrated that nimorazole improved locoregional control, but not overall survival.[9] Nimorazole is an established hypoxia modifier in Denmark but is not widely used internationally. As is the case with hypoxia-activated pro-drugs, hypoxic cell radiosensitisers require adequate delivery to areas of low oxygen tension within tumours, which are inherently poorly perfused and therefore difficult to reach.

In contrast to the aforementioned strategies aimed at alleviating hypoxia, an emerging alternative strategy is to increase the concentration of oxygen in a tumour through modulation of cellular metabolism. The hypothesis is that inhibition of oxygen consumption of cells in adequately perfused and peri-hypoxic regions allows unmetabolised oxygen to diffuse into hypoxic areas. Mathematical modelling indicates that inhibiting oxygen consumption can lead to alleviation of radiobiological hypoxia and may be more efficacious than attempts aimed at increasing oxygen supply.[10]

Oxygen is primarily consumed by mitochondria and the associated oxidative phosphorylation (OXPHOS) and electron transport chain (ETC). Well-studied ETC inhibitors such as arsenic trioxide (AsO_3) have been demonstrated to alleviate hypoxia in experimental animal models;[11] however, such compounds have significant toxicity at pharmacologically relevant concentrations thus prohibiting their clinical use. Other agents have also been known to reduce cellular oxygen consumption, such as the PI3K inhibitors BEZ235 and BKM120,[12] anti-inflammatory drugs diclofenac and indomethacin,[13] or glucocorticoids such as dexamethasone or prednisolone;[14] however, it is unclear whether many of these compounds target the ETC directly or as a weaker off-target effect. As a result, it is unclear if therapeutically relevant doses can be achieved without aberrant clinical toxicity.

Interestingly, the frequently prescribed anti-diabetic drug metformin has recently been demonstrated to inhibit OXPHOS through complex I inhibition and able to alleviate hypoxia *in vivo*.[15] Newly uncovered effects of this drug continue to emerge—Metformin has been shown to have anti-oxidant properties,[16] is capable of activating the ATM/Chk2-regulated repair pathway,[17] and inhibit the PI3K & Akt/mTOR1 pathways.[18] It may also act to couple mitochondrial bioenergetics to DNA repair and thereby influence radiotherapy efficacy in a hypoxia-independent mechanism.[19] After promising pre-clinical work, metformin is currently being investigated in a number of clinical studies, for example, in a Phase II trial (NCT02394652) for locally advanced cervix cancer patients treated with platinum-based chemoradiotherapy. If successful, it will represent one of the first examples of a proven ETC inhibitor demonstrating efficacy in a randomised clinical study. The success of metformin in alleviating hypoxia is however contingent upon adequate pharmacological concentrations being achieved in patients without significant increase in radiation toxicity or off-target effects.

Excitingly, another commonly prescribed drug, atovaquone, has recently been shown by our group to inhibit oxygen consumption and alleviate hypoxia *in vivo*.[20] This off-patent, anti-malarial agent is an ubiquinone analogue and specific complex III inhibitor demonstrated to reduce oxygen consumption *in vitro* without inducing aberrant cell death. It reliably causes a significant tumour growth delay *in vivo* when combined with radiation, and importantly, these effects are seen at plasma concentrations that are easily achievable clinically. In light of such promising results, our team is now conducting a Phase 0 proof-of-principle clinical trial [Atovaquone as a Tumour hypOxia Modifier (ATOM), NCT02628080]. This study is investigating the effect of atovaquone on tumour hypoxia in patients with resectable NSCLCnon-small cell lung cancer (NSCLC) —a classical window-of-opportunity study. If demonstrated to reduce tumour hypoxia in patients, larger clinical trials will be conducted to determine whether this well-tolerated and inexpensive agent improves radiotherapy outcomes. Of equal importance, the ATOM study also aims to validate research techniques for investigating tumour hypoxia in the clinical setting which include: [^{18}F]-fluoromisonidazole positron emission tomography (PET), perfusion CT, dynamic contrast enhanced and diffusion-weighted imaging MRI, serological hypoxia markers, resected tumour immunohistochemistry (for both endogenous and exogenous markers of hypoxia) and tumour hypoxia metagene analysis. By correlating data from this wide range of techniques, not only will a thorough investigation into the effect of atovaquone be conducted, but also it is hoped that a clinically useful hypoxia biomarker will be identified for use in future studies.

Although at an early stage in its development, this novel strategy to alleviate tumour hypoxia through OXPHOS inhibition appears very promising. This new approach represents a transition from targeting perfusion-limited hypoxic cells to targeting cells at the hypoxia-normoxia interface. It is however important to recognise that in order to realise the full potential of OXPHOS inhibition as an anti-hypoxia strategy, robust hypoxia biomarkers must be developed and incorporated in future clinical trials to identify patients who may benefit from such treatment. It is likely that only when accurate and clinically deliverable hypoxia-dependant patient stratification is possible will hypoxia modifiers finally transition into routine clinical use after more than half a century of asking.

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