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Editorial

Tumour Hypoxia

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Tumours are commonly hypoxic relative to surrounding tissues and oxygen plays an important role in radiosensitivity [1]. An understanding of hypoxia in tumours is therefore a key element of radiobiology, with hypoxia a target for improving patient outcomes. Here we discuss some of the fundamental principles related to understanding hypoxia as a therapeutic target, including the influence of oxygen on radiosensitivity, the oxygen enhancement ratio (OER), the relationship between the OER and linear energy transfer (LET) and the role of reoxygenation.

Oxygen Levels in Tissues

When we talk about oxygen in radiobiology it is in terms of oxygen tension (mmHg) or percentage oxygen concentration. The actual SI unit (Pascal) is not widely used. Oxygen tension decreases from air (160 mmHg, 21% oxygen), arterial blood (80–100 mmHg, 11–13% oxygen), venous blood (40 mmHg, 5%), normal tissues (40–60 mmHg, 5–8%) to tumours (7–28 mmHg, 1–4%). We use the words normoxia (air), physiological normoxia (normal tissue), hypoxia (low oxygen levels but not specified), radiobiological hypoxia (hypoxia associated with radiation resistance, <1 mmHg/0.13%) and anoxia (no oxygen) [2].

The Influence of Oxygen on Radiosensitivity

When ionising radiation interacts with tissue it causes excitations (electrons in atoms move from one valence shell to a higher one) and ionisations (elimination of an outer orbiting electron from an atom). Ionisations cause molecules to fragment and produce free radicals (parts of molecules with an unpaired electron). Ionisations occur throughout cells but the most prevalent radiation-induced free radical is the hydroxyl (·OH) radical (Figure 1A) because it is formed from the ionisation of water, which makes up about 80% of cells. Free radicals are highly reactive, short-lived species. As electrons like to exist within atomic shells in pairs, free radicals try to stabilise by acquiring another electron as soon as possible. Direct or indirect (e.g. ·OH induced) damage to DNA (and other macromolecules) is readily stabilised by sulphydryl groups and repaired, but in the presence of oxygen a peroxy radical is formed that 'fixes' damage into a permanent irreparable state [3]. The oxygen effect refers to the increased sensitivity of cells to ionising radiation-induced damage as oxygen concentration increases [4]. Maximal radiosensitivity occurs around 40 mmHg; half maximum at around 3–7 mmHg (0.5–1%). Oxygen must be present during or within milliseconds of irradiation.

Oxygen Enhancement Ratio and its Relationship with Linear Energy Transfer

The OER is the ratio of radiation dose in hypoxia divided by the radiation dose in air to produce the same biological effect (Figure 1B). OERs are generally between 2.5 and 3 for sparsely ionising radiation. It is less important for cell kill caused by charged particles, such as alpha radiation, that have a high LET. OER reduces at low doses and decreases with increasing LET. OER drops to 1 at around 100 keV/μm (Figure 1C) [5]. The reduced OER at low doses (<3 Gy) has been attributed to survival being dominated by cells in a radiosensitive G1 phase of the cell cycle, which have lower OERs [6]. The reduction is probably due to the low-dose response being dominated by irreparable damage (α) due to direct versus indirect radiation effects. Any implication

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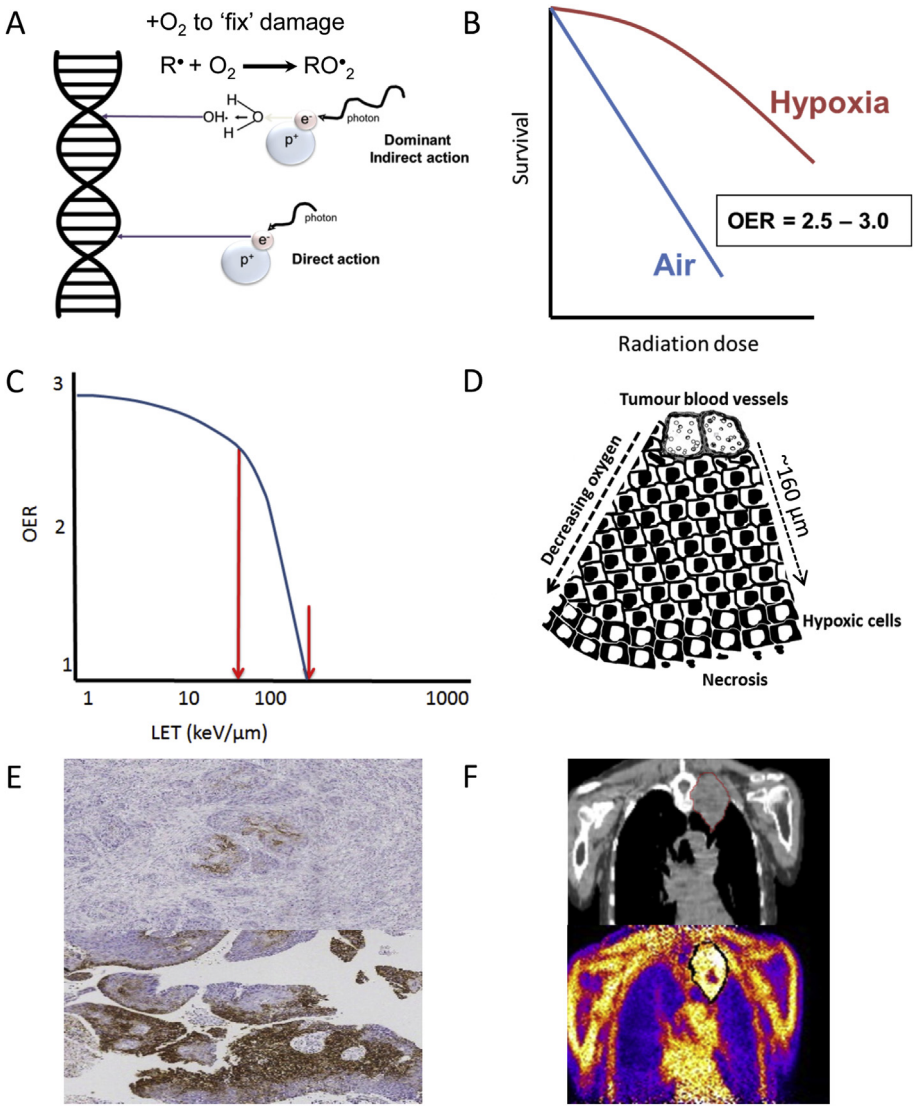

Fig 1. (A) Direct and indirect mechanisms of DNA damage by radiation (direct - direct ionisation of a DNA molecule by an incident photon; indirect - ionisation of a water molecule by an incident photon to produce a hydroxyl radical that damages DNA). Low linear energy transfer (LET) radiation interactions are predominantly via indirect ionisation, whereas high LET radiations (charged particles) produce greater DNA damage via direct ionisation. (B) The oxygen enhancement ratio (OER) for low LET radiations. Under hypoxic conditions, a greater radiation dose is required to produce the same fraction of cell kill compared with under normoxic conditions. (C) Relationship between OER and LET. At low LET, OER is close to 3. As LET increases OER decreases. OER decreases more rapidly above 60 keV/μm and reaches 1 around 100–200 keV/μm. (D) Diagram showing how hypoxia develops as tumour growth outstrips blood supply. (E) Light microscopy images of head and neck tumours stained with the hypoxia-inducible marker carbonic anhydrase IX (CA-IX) showing low and high expression. (F) Coronal computed tomography image of the thorax showing a large left apical lung tumour with corresponding ^{18}F-fluoroazomycin arabinoside (^{18}F-FAZA) positron emission tomography showing the central region of tumour hypoxia. Image reproduced with permission of Dr Ahmed Salem, University of Manchester.

for altered fractionation is not clear as the reduced OER at low doses seen in cell lines is not a universal finding [7].

Tumour Hypoxia

A seminal paper in the hypoxia field was written by Thomlinson and Gray in 1955 [8]. This study explored the histological structure of some human lung cancers and noted that there is no tumour cord >200 μm in radius without central necrosis, no central necrosis in any tumour cord <100 μm in radius and the thickness of viable tumour cells never exceeds 180 μm. The authors inferred that, as the known oxygen diffusion distance in tissue is about 160 μm, then solid tumours must contain hypoxic cells distant from the capillaries in the stroma (Figure 1D). As cells are more resistant to ionising radiation in hypoxia, it was postulated that hypoxia would limit radiocurability and should be targeted.

Hypoxia arises via different mechanisms and is often categorised as chronic/diffusion-limited or acute/transient/intermittent/perfusion-limited. A further category that is sometimes considered is intermediate/moderate hypoxia – cells that are on the way to becoming chronically hypoxic.

Cancer is characterised by uncontrolled proliferation, with tissues bypassing normal cellular homeostatic mechanisms and cell growth exceeding the requirements of normal physiology. As a result of these processes, cell proliferation exhausts the oxygen supply. Cells respond rapidly to decreased oxygen levels, and those distant from blood vessels become chronically hypoxic and die unless they migrate closer to or stimulate the formation of new blood vessels.

The main regulator of changing oxygen levels is hypoxia inducible factor (HIF)-1α [9]. Cells produce HIF-1α continually but it degrades when oxygen is present. When oxygen levels fall, HIF-1α stabilises and binds to hypoxia response elements of key genes in the nucleus, leading to their transcription. Key genes include those involved in angiogenesis (e.g. *VEGF*), the switch to anaerobic glycolysis (e.g. *GLUT1*) and pH regulation (e.g. *CA9*). Tumour cell growth leads to hypoxia-induced stimulation of angiogenesis, but the generation of poorly functioning blood vessels (they are chaotic and lack supporting pericytes). Tumour blood vessels are prone to collapse, leading to their transient occlusion and acute hypoxia. This unique characteristic of tumour vasculature led to the development of vascular disrupting agents (different from anti-angiogenic agents) as an anti-cancer strategy to combine with radiotherapy [10].

Importance of Reoxygenation

Withers [11] defined the phenomena, identified from radiobiology studies, that influence the outcome of fractionated radiotherapy as the 'four Rs'. Reoxygenation is one of these four Rs, its principal evidence base coming from animal studies of fractionated irradiation. Early radiobiology studies showed that the proportion of hypoxic cells in an animal tumour model was similar during a course of radiotherapy – if there was no reoxygenation, the proportion would increase [12]. Reoxygenation is important because hypoxic versus oxygenated cells are two to three times less sensitive to sparsely ionising radiation, and so fractionation allows radioresistant hypoxic cells to reoxygenate and become more radiosensitive. Reoxygenation is only important for tumours, as normal tissues are better oxygenated. The kinetics of reoxygenation was shown to vary across different animal tumour models [12]. There is some evidence for reoxygenation in human tumours from imaging studies [13], but it is probably variable within and between tumour types. Mechanisms of reoxygenation include: tumour shrinking due to radiation-induced cell loss during radiotherapy allowing chronically hypoxic cells to be closer to a blood supply; and increased oxygen availability due to reduced oxygen consumption when cells are killed by radiation.

Measuring Hypoxia

There are many ways to assess tumour hypoxia. Oxygen electrodes are the most direct method. The first studies involving large electrodes were reported in the 1960s, but the development of fine needle electrodes and automated insertion was important. The Eppendorf pO_2 histograph enabled a large number of measurements to be made quickly to avoid tissue compression and bleeding artefacts associated with large electrodes. Median oxygen tensions vary across different tumour types: breast (28 mmHg), sarcoma (18 mmHg), head and neck (16 mmHg), melanoma (10 mmHg), glioblastoma (7 mmHg), prostate (7 mmHg). The first Eppendorf studies provided unequivocal evidence that solid tumours were hypoxic and that hypoxia affected survival. A paper by Hockel *et al.* [14] showed that patients with carcinoma of the cervix with hypoxic tumours had significantly worse disease-free and overall survival probabilities compared with patients with non-hypoxic tumours [14]. Importantly, the study showed that hypoxia was associated with a poor prognosis, irrespective of whether surgery or radiation was the primary treatment. Oxygen electrode data showed that the level of tumour hypoxia varies between and within tumour types, and that patients with the most hypoxic tumours have the worst prognosis. The Eppendorf machine is no longer manufactured and could only be used on accessible tumours. Research has explored alternative approaches for measuring hypoxia, e.g. exogenous probes (e.g. single dose administration of pimonidazole followed by biopsy and immunostaining), endogenous markers (e.g. HIF-1α, CA9; Figure 1E), gene signatures, positron emission tomography (e.g. ^{18}F-fluoromisonidazole, ^{18}F-fluoroazomycin arabinoside; Figure 1F) and functional imaging (e.g. dynamic contrast-enhanced magnetic resonance imaging). All the examples listed have been studied in cancer patients and shown to provide prognostic information.

Evidence that Targeting Hypoxia Works

There is level 1a evidence that giving hypoxia-targeting treatment with radiotherapy improves cancer patient outcomes. A meta-analysis of 86 randomised trials involving 10,108 patients showed that giving a hypoxia-targeted treatment with radiotherapy increased locoregional control and overall survival [1]. The approaches involve increasing oxygen delivery (e.g. carbogen plus nicotinamide), hypoxic cell radiosensitisers (e.g. nimorozole) and hypoxia-specific cytotoxins (e.g. tirapazamine) [1]. The first trials used hyperbaric oxygen and hypoxic cell radiosensitisers (metronidazole, misonidazole). The radiosensitisers are electron affinic – they mimic the effects of oxygen and must be present during irradiation. The first radiosensitisers studied caused peripheral neuropathy, which led to the development of similar compounds that were less electron affinic and toxic. The DAHANCA-5 trial showed that giving nimorazole with radiotherapy to patients with head and neck cancer increased overall survival by 10% [15]. The UK BCON (bladder, carbogen and nicotinamide) trial showed that giving carbogen and nicotinamide with radiotherapy improved overall survival by 13% in patients with muscle-invasive bladder cancer [16]. The Dutch

ARCON (accelerated radiation, carbogen and nicotinamide) trial showed that hypoxia targeting improved regional control of laryngeal cancer by 7% [17]. NIMRAD is a UK trial randomising head and neck cancer patients unsuitable for concurrent chemotherapy to radiotherapy alone or with nimorazole [18]. There is good evidence that patients with the most hypoxic tumours benefit the most from having hypoxia-targeting agents with radiotherapy [19,20].

Core Learning Outcomes

- Cells are two to three times more radioresistant to sparsely ionising radiation when irradiated in the absence of oxygen.
- Oxygen fixes DNA damage by free radicals.
- The OER is the ratio of radiation dose in hypoxia divided by the radiation dose in air to produce the same biological effect.
- OER decreases with increasing LET.
- All solid tumours have hypoxia – some more than others.
- Reoxygenation occurs during a course of fractionated radiotherapy, making tumour cells more sensitive to radiation.
- Hypoxia promotes angiogenesis and metastasis – it is associated with an aggressive phenotype.
- High levels of tumour hypoxia are associated with a poor prognosis.
- Hypoxia modification strategies are effective.
- Patients with the most hypoxic tumours benefit the most from hypoxia-modifying treatments.

Hypoxia Best of Five Questions

1. Which of the following statements is the most correct regarding the presence of oxygen in irradiated cells?
    a. Oxygen acts as a radical scavenger by converting free radicals to non-reactive species.
    b. Oxygen acts as a radioprotector.
    c. Oxygen reacts with hydrogen radicals to form water, thus reducing the number of free radicals formed.
    d. Oxygen modifies the level and spectrum of free radical damage produced in DNA.
    e. Oxygen is unlikely to play a role in the indirect effect of radiation.

Answer: d. In irradiated cells, oxygen increases the number and/or type of free radicals and acts as a radiosensitiser by increasing the level of damage produced. Oxygen further reacts with free radicals, resulting in the production of different radical species, which may be longer lived and, therefore, more damaging than the original radicals. For example, oxygen may react with hydrogen radicals to produce peroxyl radicals. Through its reaction with free radicals formed from the radiolysis of water, oxygen plays a role in the indirect effect of radiation.

2. Which of the following statements is the most correct regarding tumour hypoxia?
    a. Tumours <2 mm in diameter are fully oxygenated.
    b. The diffusion distance of oxygen in tissues is around 300 μm.
    c. Only hypoxic cells survive following a single dose of radiation.
    d. Reoxygenation increases the radioresistance of tumour cells.
    e. Mechanisms of reoxygenation involve decreased cellular respiration and recirculation of blood through temporarily occluded blood vessels.

Answer: e. Tumours <2 mm in diameter will have hypoxic cells. The diffusion distance of oxygen in tissues is around 160 μm. Both normoxic and hypoxic cells may survive following a single dose of radiation. Reoxygenation increases the radiosensitivity of tumour cells. Chronic hypoxia may reduce following tumour cell loss as tumour cells may be closer to a blood vessel and there is increased oxygen availability for the remaining cells. Acute hypoxia may reduce following recirculation of blood through temporarily occluded blood vessels.

3. Which of the following statements is the most correct about the oxygen enhancement ratio (OER)?
    a. The OER is the ratio of surviving fractions produced by the same radiation doses under two different oxygen conditions.
    b. The OER is the ratio of radiation doses that produce the same surviving fractions under two different oxygen conditions.
    c. Free radical scavengers can increase OER values.
    d. High LET and high dose rate radiation may have higher OER values.
    e. The OER decreases as the radiation dose increases.

Answer: b. The OER is calculated based on the ratio of doses, not the effect. Free radical scavengers will reduce the contribution of the indirect effect, thus reduce OER. High LET and high dose rate radiation often have a lesser reliance on indirect ionisation for cell kill and should have lower OER values.

4. Which of the following has not been used to assess hypoxia in human tumours?
    a. An X-ray angiograph.
    b. The Eppendorf pO_2 histograph.
    c. Pimonidazole staining.
    d. ^{18}F-FMISO positron emission tomography.
    e. Expression of hypoxia-inducible proteins, for example CA-IX.

Answer: a.

5. Which of the following might be given with the intention of improving the availability of oxygen in a tumour?
    a. Cisplatin.
    b. Erythropoietin.
    c. Mitomycin C.
    d. Prednisolone.
    e. Tirapazamine.

Answer: b.

6. Which of the following statements about hypoxic cell sensitisers and cytotoxins is the most correct?
    a. Hypoxic cell radiosensitisers increase the radiosensitivity of oxygenated and hypoxic cells.
    b. Bioreductive drugs selectively kill hypoxic cells and examples are tirapazamine, mitomycin C and cisplatin.
    c. Hypoxic cell radiosensitisers such as nimorazole are electron affinic compounds that mimic the sensitising effects of oxygen.
    d. Breathing high oxygen concentrations is used to increase the oxygen carrying capacity of blood.
    e. ARCON stands for Accelerated Radiation, Carbon dioxide and Nicotinamide.

Answer: c. Hypoxic cell radiosensitisers increase the radiosensitivity of hypoxic cells not normoxic cells. Bioreductive drugs selectively kill hypoxic cells and examples are tirapazamine and mitomycin C not cisplatin. Breathing high oxygen concentrations is used to increase the amount of oxygen delivered to a tumour. ARCON stands for Accelerated Radiation, Carbogen and Nicotinamide. Carbogen is 95% oxygen and 5% carbon dioxide.

Conflicts of Interest

The authors declare no conflicts of interest.

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