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EDITORIAL

Cellular and Molecular Biology

Elucidating the role of transiently hypoxic tumour cells on radiation resistance

The link between hypoxic conditions and radiation sensitivity is well-established, however the dynamic nature of hypoxia is often overlooked. The contribution of acute/transient hypoxia versus chronic conditions to radiosensitivity has been investigated by Wadsworth et al. using two hypoxia markers and pentoxifylline to increase blood flow to regions of transient hypoxia.

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Regions of low oxygen in solid tumours have long been described as either acutely or chronically hypoxic. Equally established is the dynamic nature of tumour hypoxia and specifically that cells in regions of acute hypoxia experience transient periods of hypoxia followed by reoxygenation and/or become chronically hypoxic [1]. Our understanding of hypoxia-mediated therapy resistance has been limited by technological challenges in determining the relative contributions of acute/transient versus chronic hypoxia in vivo. Here, Wadsworth et al. have used the sequential injection of two hypoxia markers (pimonidazole and EF5) to label both chronically and acutely/transient hypoxic cells in xenograft tumours [2]. They focus on two cell lines, one of which, a colon adenocarcinoma, is prone to dynamic hypoxia while the other, a cervical squamous carcinoma, experiences more stable hypoxia when grown in vivo [3]. When the hypoxia markers were co-administered both tumour types were 30–40% hypoxic with an almost complete overlap in pimonidazole and EF5 staining confirming that the drugs are equally tumour penetrant and reduced at similar oxygen tensions. To explore the dynamic nature of tumour hypoxia, the timing between the injection of pimonidazole and EF5 was extended and demonstrated that, as this interval increased so did the population of cells positive only for EF5. Using this approach, they demonstrated that pimonidazole positive cells were lost over time and that this occurred in part due to them being pushed into the necrotic regions of the tumour. Importantly, they verified that the loss of pimonidazole positive cells could not be solely attributed to the proliferation of labelled cells and consequent dilution of the pimonidazole adducts in daughter cells. Given that the total hypoxic fraction did not change during the experimental timeframe, these data suggest that cells become newly hypoxic (EF5 positive) while the previously hypoxic cells (pimonidazole positive) die. To investigate the relative proportion of transient and chronic hypoxia, pentoxifylline (a phosphodiesterase inhibitor licensed for the treatment of peripheral vascular disease) was used to improve red blood cell deformability and increase flow through the constricted tumour vasculature. The use of pentoxifylline leads to the reoxygenation of regions experiencing transient hypoxia but has no impact on the chronically hypoxic regions (Fig. 1) [3]. Once able to distinguish between transient and chronic hypoxia [4], Wadsworth et al. determined the half-lives of cells in both conditions and found that while the cells in chronic hypoxia have a constant rate of death but can survive beyond 96 h, those experiencing transient hypoxia do not start to die until after 24 h but were no longer detectable by 72 h after labelling. The data presented suggest that the transiently hypoxic cells both die in situ as well as being pushed into chronically hypoxic regions and eventually undergoing necrosis. This, in turn, raises the question of the mechanism of cell death in transient hypoxia and specifically why this appears to happen faster than observed in chronic hypoxia. Notably both cell lines studied lack functional p53, through HPV-mediated degradation or because of mutation, which if present would be expected to drive hypoxic cells into apoptosis and decrease their half-life in vivo [5]. The likely presence of increased reactive oxygen species and DNA damage in the hypoxic regions also experiencing periods of reoxygenation likely contribute to the faster turnover of transient hypoxic cells compared to the chronically hypoxic [6].

Numerous studies have shown that tumour hypoxia is a major barrier to radiation-induced cell killing and ultimately patient response to radiotherapy [7]. Wadsworth et al. went on to use their models to ask how cells in transient hypoxia respond to radiation. To do this they used three different radiation protocols, 5 Gy, 10 Gy or 2×5 Gy (with an interval of 6 h) and again employed pentoxifylline to determine the transient component of the irradiated tumours. In each of the three radiation protocols, the radiation-induced cell killing was reduced by the addition of pentoxifylline indicating that the transiently hypoxic fraction of the tumours contributes to hypoxia-mediated radiation resistance. In response to the single large dose of radiation (10 Gy) the impact of pentoxifylline on radiosensitivity was lost (at the longer time point) which was attributed to vascular disruption. The data supports the conclusion that by disrupting the vasculature the transiently hypoxic cells, which tend to be in close proximity to the vasculature, are lost and therefore tumour radiosensitivity increases. It would be interesting to determine how the proportion of transiently hypoxic cells alters during a course of fractionated radiotherapy.

The conclusion that the transient hypoxic cells are equally radiation resistant as the chronically hypoxic raises several interesting questions. For example, previous studies have concluded that

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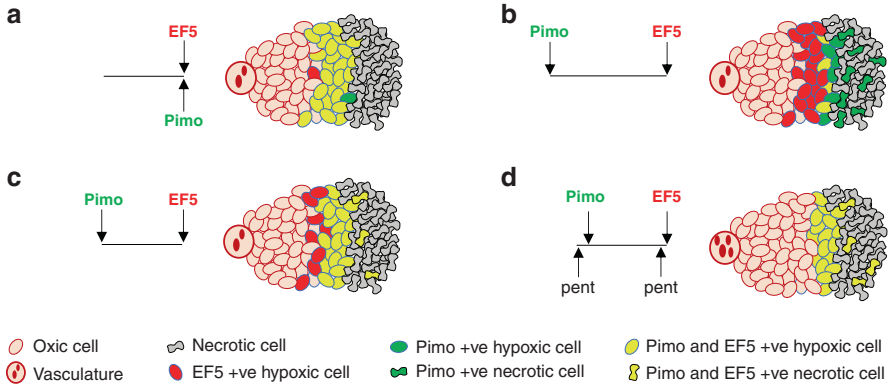

Fig. 1 Distinguishing transient and chronic hypoxia in vivo using EF5, Pimonidazole and pentoxifylline. In each schematic the relative timing of administration of the drugs are indicated. **a** EF5 and Pimonidazole (Pimo) were co-administered and identified the hypoxic fraction, which was almost exclusively positive for both hypoxia markers. **b** In contrast, when the administration of the two markers was separated by 72 h there was little co-staining and an evident Pimo positive necrotic population. **c** The interval between administration of the hypoxia markers was reduced to 24 h, here co-stained hypoxic and necrotic cells were evident as well as an EF5 only population. **d** When the conditions shown in (**c**) were repeated with the inclusion of pentoxifylline (pent) to increase blood flow and reoxygenate transiently hypoxic cells prior to Pimo or EF5 injection, the remaining Pimo and EF5 labelled cells represented chronically hypoxic cells.

cells experiencing cyclic hypoxia are more radiation resistant than those in chronic conditions although notably some of these studies have included periods of non-physiological reoxygenation as part of the cycling parameters (reviewed in ref. [1]). Pimonidazole and EF5 report on oxygen levels below 10 mmHg (1.3% O_2) [8] which means that all cells experiencing oxygen levels between 0 and 1.3% will be pimonidazole/EF5 positive but the oxygen enhancement ratio (OER) of these cells will differ significantly [9]. It seems likely that oxygen levels will be lower in the chronically hypoxic region compared to during transient hypoxia and, therefore, if radiation resistance is similar in these two regions it likely infers contributing factors in the biological response to transient hypoxia.

A current limitation in the hypoxia field is the challenge of determining actual oxygen level as opposed to using reporters of a relatively broad range of conditions. Currently, assessing hypoxia clinically is restricted to the use of PET to detect agents, which likely detect chronic hypoxia, such as FMISO and FAZA [10]. The data presented highlight an unmet need to study transient hypoxia both experimentally to determine the relevant parameters, including time and oxygen level, as well as to develop technologies capable of identifying patients with tumours with a high transient hypoxia fraction. If the data presented are representative of clinical scenarios, they suggest a significant fraction of tumours experience transient hypoxia (up to 26% in this model), that transiently hypoxic cells behave aggressively after irradiation and that our current methods may well be underreporting the hypoxic nature of specific tumours. Together, these findings suggest new approaches/technologies are required to reliably stratify patients for example in studies involving dose escalation or hypoxia activated prodrugs.

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GSH and EMH co-wrote the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

No ethical approvals were required for this work.

CONSENT TO PUBLISH

Not applicable.

ADDITIONAL INFORMATION

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