

REVIEWS

Understanding hypoxia microenvironment of micro-metastases

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Abstract

Most cancer-related deaths are due to the development of metastatic disease rather than the growth of primary tumors. Adjuvant treatment often does not translate into substantial improvements in overall survival; subclinical micro-metastases may be resistant to multiple therapies. Understanding microenvironment factors such as hypoxia, proliferation and glucose metabolism in micro-metastases is of importance for micro-metastases treatment; hypoxia, commonly observed in most primary solid malignancies, is associated with tumor progression, increased aggressiveness, enhanced metastatic potential and poor prognosis and hypoxic tumor cells are more resistant to radiotherapy and some forms of chemotherapy. In this article, we discussed hypoxia status of micro-metastases, and related this to cellular proliferation and glucose metabolism. We also proposed hypoxia as a therapeutic target for micro-metastases.

Key words

Hypoxia, Micro-metastasis, Proliferation, Glucose metabolism, Hypoxia targeted therapy

1 Introduction

Most cancer-related deaths are due to the development of metastatic disease rather than the growth of primary cancer. The prevention of this development or the elimination of metastases before they become clinically detectable would be expected to result in improvements in cancer mortality rates. Adjuvant treatment in the form of systemic chemotherapy and/or local-regional radiotherapy is generally given after surgical removal of the primary cancer. This often does not translate into substantial improvements in overall survival ^[1].

Hypoxia is a common feature of primary solid malignancies and the presence of hypoxia is recognized as an important determinant of clinical outcome ^[2-6]. We have shown, in an animal model of colorectal cancer metastases in the peritoneal cavity, that microscopic tumors of less than 1 mm diameter were extremely hypoxic while those of greater size (1 - 4 mm diameter) were not significantly hypoxic ^[7-10]. In contrast, other group has found hypoxia presented in metastases as they grew larger than 2 to 3 mm in diameter; however, hypoxia status in sub-millimeter metastases has not investigated and reported in this study ^[11]. Recent review articles have discussed hypoxia status in metastases ^[10, 12]. Here, we extended the

discussion on the following aspects: Hypoxia status in micro-metastases, its relation to cellular proliferation, glucose metabolism, in addition, we discussed potential therapeutic strategies toward curing micro-metastases.

2 Hypoxia in micro-metastases

The presence of hypoxia is a common feature of primary solid malignancies [2-4, 6, 10, 13]. We have recently observed on the hypoxic status of microscopic tumors established intraperitoneally and intradermally using the HT29 and HCT8 colorectal cancer lines [7-9], NSCLC A549 and HTB177 cells [14], and breast cancer MDA-MB-231 cells (Li et al., unpublished observation) using pimonidazole immunohistochemical staining. In general, sub-millimeter tumor deposits of HT29 and HCT8 showed intense hypoxia (hypoxic fraction as high as 90%) with little or no blood perfusion. Tumors ranged 1 - 4 mm in diameter seemed relatively well vascularized, well perfused and generally displayed little hypoxia. In tumors larger than 4 mm diameter, hypoxia reappeared in the characteristically perinecrotic distribution pattern seen in macroscopic tumors [7]. We have also observed similar patterns of tumor hypoxia in experimental lung metastases of A549 as well as in liver and kidney metastases of MDA-MB-231 cells (Li et al. unpublished observation). Severe hypoxia may be a general feature in micro-metastases. Future studies are needed to confirm whether the pattern of severe hypoxia of micro-metastatic diseases found in mouse models can apply to patients.

3 Hypoxia and proliferation

Sub-millimeter metastases may have already existed in many patients when primary cancers were initially diagnosed although without clinical evidence of distant metastases. Human sub-millimeter metastases may be avascular and in a state of dormancy (i.e. non-expanding in mass) [15, 16]. Cell proliferation in dormant tumors had been observed, but results were mixed: Cells were either dividing very slowly or were in G0 phase [17-20], others have found proliferation in dormant tumors to be as high as in macroscopic vascularized tumors but that the dormant tumors did not grow beyond a threshold size due to a kinetic balance between proliferation and apoptosis [21, 22]. Hypoxia status had not observed in dormant micro-metastases. In animal model of metastases, we have noted that cellular proliferation were in the non-hypoxic rim but not the interior hypoxic core of sub-millimeter avascular tumors, whereas proliferating cells were found throughout larger tumors 1 - 4 mm in diameter which were less hypoxic [7-10]. This is in good agreement with several studies that cellular proliferation and hypoxia are mutually exclusive in macroscopic tumors [14, 23-25]. Future studies would confirm whether cancer cells in dormant metastases are proliferative, this concept is very important for systemic chemotherapy of metastases; chemotherapy generally kills proliferating cancer cells.

4 Hypoxia and angiogenesis

Hypoxia has been recognized as a primary physiological regulator of angiogenesis [26, 27]. Our results suggest the possibility that the existence of severe hypoxia in microscopic tumors could be common irrespective of cell line and tumor location and reflects the pre-angiogenic stage of tumor development. As peritoneal and intradermal tumors increased in size to the diameter range 1 to 4 mm, there was a drastic reduction in tumor hypoxia coupled with the appearance of functional tumor vascularization [7-9]. This suggests the sequence of events are that cells become hypoxic when tumors reach several hundred micrometers, hypoxia drives angiogenesis, previously hypoxic cells become oxygenated and the neovascularized tumors grow beyond the size threshold. The timing for hypoxia driving angiogenesis switch is critical important for anti-angiogenesis therapy.

5 Hypoxia and glucose metabolism

Hypoxic cells undergo a switch from aerobic to anaerobic glucose metabolism. Glycolysis may be assessed functionally by examining the uptake of ^{18}F -fluorodeoxyglucose (^{18}F -FDG). ^{18}F -FDG is an analog of glucose, and like glucose, is

phosphorylated by hexokinase, but, unlike glucose, is not metabolized further. Since the phosphorylated metabolite is unable to leave the cell, the intracellular accumulation of ^{18}F -FDG may be assayed. In vitro experiments show that incubation in hypoxic conditions induces an increase in cellular FDG uptake [28-30]. It was recently shown that the intratumoral distribution of ^{18}F -FDG in R3327-AT rat prostatic carcinoma xenografts positively correlated with that of the hypoxic marker pimonidazole [23].

We have observed glucose uptake in microscopic tumors grown intraperitoneally in nude mice using ^{18}F -FDG digital autoradiography and to relate this to physiological hypoxia and glucose transporter-1 (GLUT-1) expression [8]. Human colon cancer HT29 and HCT-8 cells were injected intraperitoneally into nude mice to generate disseminated tumors of varying sizes. Following overnight fasting, animals, either breathing air or carbogen (a gas mixture of 95% O_2 and 5% CO_2), were intravenously administered ^{18}F -FDG together with the hypoxia marker pimonidazole and the cellular proliferation marker bromodeoxyuridine one hour before sacrifice. Hoechst 33342, a perfusion marker, was administered one minute before sacrifice. Following sacrifice, the intratumoral distribution of ^{18}F -FDG was assessed by digital autoradiography of frozen tissue sections. This was compared with the distributions of pimonidazole, GLUT-1 expression, bromodeoxyuridine and Hoechst 33342 as visualized by immunofluorescent microscopy.

We found that small tumors (< 1 mm diameter) had high ^{18}F -FDG accumulation and were severely hypoxic with high GLUT-1 expression and low proliferation. Hypoxia results in up-regulation of glucose transporters and hexokinase proteins [31-35], key facilitators of glucose uptake and metabolism. In addition, anaerobic glycolysis is an inefficient biochemical pathway of energy generation, requiring significantly more glucose molecules than oxidative phosphorylation to produce similar amounts of ATP. These factors may be related to the higher uptake of ^{18}F -FDG in hypoxic cells. Larger tumors (1-4 mm diameter) generally had low ^{18}F -FDG accumulation and were not significantly hypoxic with low GLUT1 expression but high proliferation. Interestingly, carbogen breathing significantly decreased ^{18}F -FDG accumulation and tumor hypoxia in microscopic tumors but had little effect on the level of GLUT-1 expression. We concluded that micro-metastases have high ^{18}F -FDG uptake, therefore, high glucose demand, which is spatially associated with physiological hypoxia and high GLUT-1 expression. This enhanced uptake was abrogated by carbogen breathing, indicating that in the absence of physiological hypoxia, high GLUT-1 expression, by itself, was insufficient to ensure high ^{18}F -FDG (glucose) uptake [8].

Aerobic glycolysis, the so-called “Warburg effect” [36] thought to be a fundamental feature of cancer [37]. In operational terms the existence of aerobic glycolysis would confer a general increase in ^{18}F -FDG uptake throughout tumors, spatially unrelated to the micro-distribution of hypoxia. It would thus result in a relatively high ^{18}F -FDG “background”. However, it should also be noted that ^{18}F -FDG uptake in non-hypoxic regions of cancer or metastases was significantly lower than that in hypoxic cancers [8, 14, 23] and is not statistically different from stromal or necrotic regions (Li et al. unpublished data). Apparently, glucose demand measured by ^{18}F -FDG is heterogeneous in cancer cells of tumors, and largely depends on hypoxia status; this is hard to fully explain by “Warburg effect”.

6 Novel therapeutic strategies for micro-metastases

Micro-metastases are severe hypoxic, the efficacy of adjuvant/neoadjuvant treatments in the form of chemotherapy and/or radiotherapy may be compromised by hypoxic resistance [7, 10]. If this is true, then new strategies will be required to meet the challenge, possibly with the aim of converting hypoxia into a target for systemic therapies. The high demand for glucose displayed by hypoxic micro-tumors [8] suggests that glucose metabolism may be a suitable target for developing novel therapies for micro-metastatic disease. Several therapeutic strategies are under investigation to exploit or interrupt tumor glycolytic metabolism [38, 39]. Future studies to test the therapeutic efficacy of targeting glucose metabolism in micro-metastases.

There are a variety of proposed methods for targeting the different manifestations of the hypoxia phenotype that may be relevant in this context ^[40, 41]. Preliminary studies with the hypoxia-selective cytotoxin tirapazamine were encouraging ^[42-44], however the experience in large-scale clinical studies has been disappointing to date ^[45]. Alternative hypoxic cytotoxins that may represent improvements on tirapazamine are in earlier stages of investigation ^[46-48]. These types of drug rely on the hypoxia-induced bio-reduction of a pro-drug to an active form. Other groups are investigating the potential of recombinant anaerobic bacteria ^[49, 50] that become active only in regions of very low PO_2 and may be directly oncolytic or vectors for the delivery of therapeutic genes. Another possibility is to target the HIF-1 signal transduction pathway and a variety of approaches aimed at either inhibition of HIF-1 activation or HIF-1 target genes are under investigation ^[41]. To this list, one could speculatively add the possibility of a therapeutic variant of a hypoxia imaging tracer. For example, a hypoxia-selective molecule labeled with an alpha-particle emitting radionuclide with a relatively short half-life may be attractive since the cytotoxic effect of alpha-particles is not modified by the absence of oxygen ^[51]. Of course the utility of such an agent would depend on its bio-distribution and comparative dosimetry. This suggests there could be particular applicability to disseminated disease in confined body regions, such as the peritoneal cavity, where there would be restricted transfer to the systemic circulation.

7 Conclusions

Sub-millimeter metastases are intense hypoxia and have high glucose demand. Future studies should examine the hypoxic status of microscopic tumors for a range of cancer cell lines of different origins in a range of anatomical sites and, wherever feasible, in patient derived material. Hypoxia may be a potential therapeutic target of micro-metastases.

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Conflict of interest

The author declares that there is no conflict of interest statement.

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