Comparative Biochemistry and Physiology, Part B 140 (2005) 233–239

CBP

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Metabolic depression: a response of cancer cells to hypoxia?☆

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Received 4 August 2004; received in revised form 8 October 2004; accepted 12 October 2004

Abstract

Hypoxic tumours have the worst prognosis because they are the most aggressive and the most likely to metastasize. This may be because these aggressive cancers have a hypoxic core which generates signals that activate angiogenesis which enables the supply of nutrients and oxygen to a rapidly growing outer oxidative shell. The hypoxic core is a crucial element of this hypothesis, as is the fact that the cells in the hypoxic core are inherently adapted to survive hypoxia. We reasoned therefore that cancer cells exposed to hypoxia/anoxia should show the hallmarks of adaptation to hypoxia/anoxia, i.e. a down-regulation of protein synthesis and a reverse Pasteur effect. We tested this hypothesis in transformed (MCF-7) and normal (HME) human mammary epithelial cells, by exposing both cell types to a range of oxygen concentrations, including anoxia. We find that indeed protein synthesis is down-regulated in the MCF-7, but not in the HME cells in response to anoxia. The data on glycolysis are not as clear-cut, but in the light of similar previous measurements on hypoxia-tolerant animals, is still consistent with the hypothesis.

Keywords: Protein synthesis; Cancer; Anoxia; MCF-7; HMEC; Metabolic depression

1. Introduction

Solid tumours have hypoxic regions. A compilation of the data up to 1998 (Vaupel et al., 1998) shows that in human primary tumours the microcirculation is compromised, and the pO_2 of the tissue is low, linked to size (a tumour with a volume of 2 mL is extremely hypoxic), often close to 0 mm Hg, and heterogeneous. Metastatic lesions are generally similar, but may be even more hypoxic. In recurrent tumours, there is a higher proportion of the tumour that is hypoxic compared to the respective primary tumour. More recent studies have confirmed these observations (Braun et al., 2001).

This hypoxic state has two major ramifications. First, hypoxia has implications for cancer treatment. Hypoxic cells are radiation-resistant (Brown, 1999; Movsas et al., 2002) and disease-free survival time after radiotherapy is related to the pO_2 of the tumour. This has led to various treatment strategies such as attempts to oxygenate tumours during radiation, and the search for compounds that are converted to toxic radicals only under hypoxic and reducing conditions (Brown, 1999; Vaupel and Hockel, 2000).

Second, radiation therapy aside, patient survival is directly related to the average pO_2 of the tumour. But paradoxically, survival is inversely related to the degree of tumour angiogenesis, a process that should lead to an increase in the pO_2. It turns out that the most aggressive fast-growing cancers with the poorest prognoses are hypoxic, they are synthesising the hypoxia-inducible factor (HIF-1α), stimulating the production of vascular endothelial growth factor (VEGF) and promoting angiogenesis (see

Abbreviations: EDTA, ethylenediaminetetraacetic acid; FCS, fetal calf serum; HIF, hypoxia-inducible factor; HME, human mammary epithelial; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; VEGF, vascular endothelial growth factor.

☆ This paper is dedicated to the late Peter Hochachka, who instilled the value of comparative biochemistry in one of the authors (MG, now retired). It is fitting that MG's last biochemical publication embodies one of Peter's many talents, i.e. applying the lessons learned from comparative studies, to the study of human biochemistry and physiology.

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doi:10.1016/j.cbpc.2004.10.005

references in Guppy, 2002). A hypothesis to explain this seemingly contradictory state has recently been proposed by Guppy (2002). The suggestion is that the initial angiogenesis of the tumour enables the outer layer of the tumour to increase its oxidative metabolism and to proliferate, and that it does so at the expense of the core which remains hypoxic, but not necrotic. The core continues to express HIF-1α, VEGF, and all the other proteins whose genes are targets of HIF-1α, while the outer layer is perfused and grows rapidly.

An integral part of this paradigm is that the cells that eventually comprise the hypoxic core are capable of adapting to hypoxia, rather than becoming necrotic. The only way that a cell can continue to support energy-utilising processes when oxidative phosphorylation is compromised during hypoxia is to up-regulate glycolysis. This is termed the Pasteur effect, and is an effective adaptation to short-term hypoxia/anoxia. However, due to the large amounts of fuel required by this pathway, and the toxicity of the end-products, this strategy is not feasible for long-term hypoxic or anoxic insults. Instead, the strategy that is adopted by animals that survive chronic hypoxia and anoxia as a regular phase of their lifestyle (such as carp, turtles and brine shrimp) is metabolic depression. These animals coordinately depress energy production and consumption (Hochachka and Lutz, 2001; Guppy and Withers, 1999).

So in terms of energy production, in these animals there is no Pasteur effect. There is no compensation for the down-regulation of mitochondrial ATP production, glycolysis is actually down-regulated, a phenomenon termed the reverse Pasteur effect. The mechanisms involved are phosphorylation of glycolytic enzymes, regulation of glycolytic enzymes by fructose-2,6-P_2, and glycolytic enzyme association (Guppy et al., 1994; Guppy and Withers, 1999; Guppy, in press). In terms of energy consumption, there are many processes that could putatively be down-regulated during metabolic depression, and probably are. However, of all those that have been investigated, the data on protein synthesis are the most consistent. Protein synthesis accounts for a significant portion (18–30%) of the energy consumption of a cell and is down-regulated in all metabolically depressing animals in which it has been studied (Guppy and Withers, 1999; Pakay et al., 2002).

The hallmark responses therefore, of a cell that is adapted to depress metabolism in response to long-term hypoxia/anoxia are a reverse Pasteur effect and a down-regulation of protein synthesis. The question we ask in this study is whether these hallmarks are shown by the transformed cells that form solid tumours, and which may (as discussed above) be pre-adapted to survive hypoxia/anoxia. Transformed and normal cells were incubated at different oxygen tensions and rates of protein synthesis and lactate production were measured. The phosphorylation status of the alpha subunit of the initiation factor eIF2 was also measured as a possible regulator of the rate of protein synthesis. The transformed cell we used is the MCF-7 breast cancer cell line, an epithelium-derived line that is representative of a significant proportion of breast cancers. As a non-transformed comparison, we used a primary preparation of human mammary epithelial cells.

2. Materials and methods

2.1. Materials

2.1.1. Gas mixtures

Gas mixtures were purchased from BOC Gases Australia.

2.1.2. Protein synthesis

L-[2,6-^{3}H]phenylalanine (specific activity 54.0 Ci/mmol, 1 mCi/mL) and NCS II tissue solubilizer were purchased from Amersham Life Science (Buckinghamshire, England).

2.1.3. Western blotting

Rabbit anti-eIF2α(P) was purchased from Research Genetics (Huntsville, AL, USA). Sheep anti-rabbit IgG horseradish peroxidase was purchased from Silenus (Melbourne, Australia). SuperSignal West Femto Maximum Sensitivity Substrate was purchased from Pierce (Rockford, IL, USA).

2.1.4. Cells

The cells used were MCF-7 cells (ATCC number HTB-22) within 30–40 passages of purchasing, and human mammary epithelial cells (HME cells) (BioWhittaker, USA) which are guaranteed for 15 doublings.

2.2. Cell maintenance

MCF-7 cells were maintained in T75 culture flasks at less than 90% confluence, in DMEM with the following additions: 6 mM glucose, 1.5 mM glutamine, phenol red (15 mg/L), Penstrep (50 units (or μg)/L) and 5% foetal bovine serum (FBS). MCF-7 cells were harvested at a confluence of 70–80%, which occurred normally after 5 days. The cells were resuspended with a 10-mL syringe and an 18-gauge needle and seeded into the glass incubation chambers in MCDB-170 medium. For a description of the glass chambers and associated experiments, see Guppy et al. (1997, 2002). HME cells were not maintained in culture, but seeded directly from a frozen vial into the glass incubation chambers.

2.3. Seeding of cells into glass chambers

For each experiment, four air-tight glass chambers were seeded with either MCF-7 cells or HME cells. For the MCF-7 cells, the chambers were pretreated with 5 mL FBS for 5 min at 37 °C (the FBS was then removed). Two million cells were seeded into each chamber which contained 50 mL of warmed MCDB-170 medium. The