Hypoxia and Elevated Interstitial Pressures Induce Tumor Cell Proliferation

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Introduction: The solid tumor microenvironment can vary with respect to interstitial fluid pressure levels (IFP), degree of oxygen saturation, and pH. While most normal tissues cannot endure nutrient deficient states, it is known that most solid tumors can persist and sometimes proliferate under elevated interstitial fluid pressure. Such extracellular tension impedes blood flow leading to further hypoxia, subsequent acidosis, and poor delivery of chemotherapy and response to radiotherapy. Certain tumor cell lines are known to increase proliferation under hydrostatic pressure(1,2,3). It is not known if this is a universal phenomenon, nor how the physical signal is transduced. Meanwhile osteoblastic cells show reduced proliferation when cultured under hypoxic conditions(4). Using a novel pressurized cell culture system, we investigated the influence of hydrostatic pressure and hypoxia on tumor population dynamics and cell cycle state. We hypothesize that hydrostatic pressure and hypoxia will enhance cell proliferation in a cell-line specific manner.

Materials and Methods: Cell culture system. All experiments were performed using the Opticell (Biocrystal, Westerville, OH) cell culture system which was modified to permit cells to grow in a hydrostatic pressurized environment(2,3). The cells were grown as monolayers and pressurized using a fluid column adjusted to 50 mmHg of hydrostatic pressure. All pressures were confirmed using a pressure gauge (Control, Friendswood, TX). Cells for hypoxic conditions were cultured in an oxygen controlled incubator (NU-4950, NuAire, Plymouth, MN). Two human tumor cell lines, U2OS osteosarcoma and H1299 lung non-small cell lung cancer were purchased from ATCC (Manassas, VA) and grown in ATCC recommended media. Total viable cell count. Both cell lines were seeded with 1.5x105 cells per cassette and incubated for 24 hours to allow for cell attachment. At that point, three cassettes were set at each of the following experimental growth conditions: a) anoxic conditions (20% oxygen) with no hydrostatic pressure (0 mmHg); b) anoxic conditions (20% oxygen) and hydrostatic pressure (50 mmHg: the highest pressure observed in clinical studies); c) hypoxic conditions (2% oxygen) with no hydrostatic pressure; d) hypoxic conditions (2% oxygen) and hydrostatic pressure (50 mmHg). Total viable cell counts were measured after incubation times of 0, 24, and 48 hours. The 24 and 48 hour studies were done independently because by opening the hypoxia incubator, the cells would be affected by changes in atmospheric oxygen content. Cells were released from culture with trypsin-EDTA and total viable cell counts were determined using a hemacytometer. Cell counts were normalized to the 0 hour cell count. Cell cycle analysis. Following 24 hours of incubation, the cells were prepared for cell cycle analysis. After they were released from culture, they were fixed in 70% ethanol and then DNA labeled with PI/RNase staining buffer (BD Biosciences, Franklin Lakes, NJ). Cell cycle parameters were measured by flow cytometry using the FACSCalibur System (BD Biosciences, Franklin Lakes, NJ). Cell cycle analysis, using FowJo 4.3.1 (Tree Star, Ashland, OR), was performed by investigating the FL2-A profile of the cells identified to be in cycle. The cells in the S and G2 phase were considered to be in the proliferative state. Each data point represents the mean of three separate cassettes. All data analysis was performed on Microsoft Excel.

Results: Total viable cell counts: Figure1. A. In the U2OS cell line, there was no significant increase of viable cell counts induced by hypoxia and/or pressure. B. In the H1299 cell line, there was significant increase of viable cell counts induced by hypoxia ($p<0.005$) and hypoxia with pressure ($p<0.001$), but no change with pressure.

Figure 1.A. Total Viable Counts of U2OS Cells

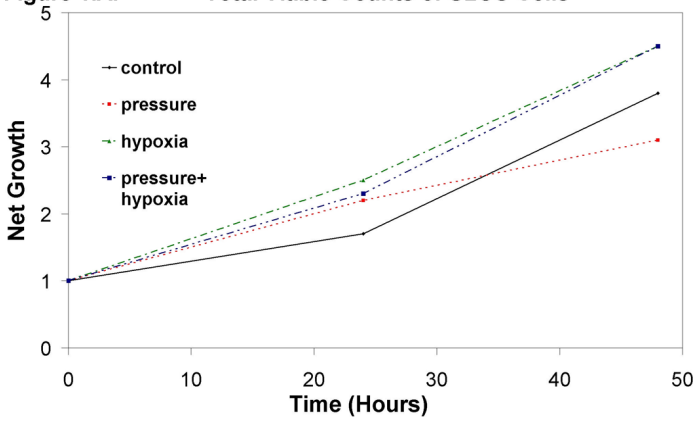
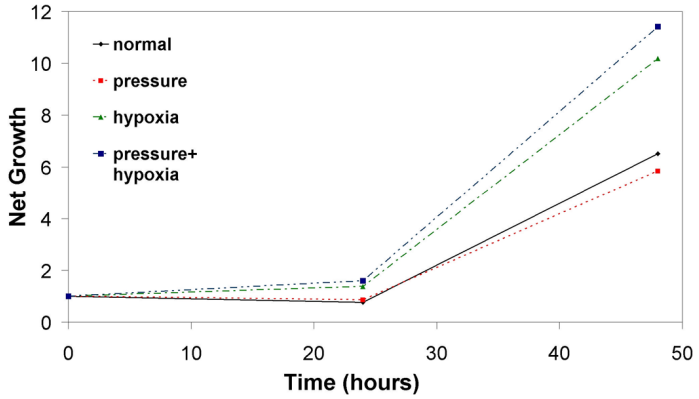


Figure 1.B. Total Viable Counts of H1299 Cells



Cell Cycle Analysis: In the U2OS cell line, there was an increase of numbers of cells in the proliferative state induced by pressure ($p<0.03$) and hypoxia with pressure ($P<0.005$). There was no significant change when induced with hypoxia only. In the H1299 cell line, there was no significant change to the proliferative state fraction.

Discussion: Hypoxia and hydrostatic pressure have variable cell specific physiologic effects. In U2OS cells, hypoxia and pressure induced no significant increase in total viable cell counts, but hypoxia and pressure induced an increase of cells in the cell proliferation fraction. In the H1299 cells, hypoxia induced a significant increase in total viable cell counts, but had no effect in the cell proliferation fraction. Hypoxia and hydrostatic pressure are toxic to normal and tumor cells, but in vivo tumor cells undergo adaptive changes that allow them to survive and proliferate(5). One way the cells adapt to the changes is through the hypoxia-inducible transcription factor 1 (HIF-1). We hypothesize that HIF-1 or a novel protein is regulating the proliferation. Overall, our findings show that tumor cells have an enhanced proliferation under hypoxic and pressurized conditions.

References: References:

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