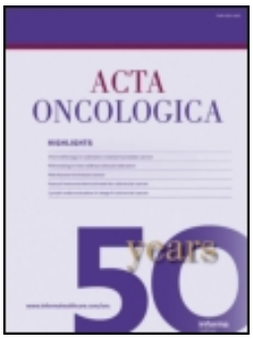

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Linking Measured Intercellular Oxygen Concentration to Human Cell Functions

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To the Editor

The cell responses to a specific pericellular oxygen concentration depend on a number of factors including cell type, duration of the particular oxygen concentration, cell cycle stage, pH and concentration of nutrients and regulatory molecules.

The isolated effect of low oxygen, however, seems decisive enough to allow some general statements about cell regulatory responses to various pericellular oxygen concentrations.

Quantification of oxygen tension around the cells is therefore an important indicator of the cell phenotype (1).

The developing understanding is that responses to hypoxia are orchestrated by a number of overlapping regulatory processes. These processes result in a sequence of changes in the cell phenotype according to changes in the oxygen concentration.

Reliable oxygen measurements in both in vitro model systems (2–5) and in vivo (6–9) are indicative of which phenotype the cell will exhibit within a given range of oxygen concentrations. The present short survey deals with these relationships.

When the intercellular oxygen concentration in tissues is reduced from normoxia (4–1% O_2 ≈4–1 kPa and corresponding to 40 000–10 000 ppm) to *moderate hypoxia (1–0.1% O_2 and kPa, corresponding to 10 000–1 000 ppm)*, respiration, and as a result the ATP production, is largely unaffected (2, 10). For a long time, this observation has stood in the way of realizing that this is not the case for many other cell functions. In fact, there is a striking effect on gene expression due to transcription, mRNA stability, protein synthesis, and post-translational modifications.

In the range of 0.1–1% O_2/kPa O_2 (1 000–10 000 ppm O_2), the central hypoxia-responsive pathways of the hypoxia-inducible factor (HIF) are functionally very important. These pathways function by binding to hypoxia-responsive elements on promoters of more than 60, but not all, hypoxia-regulated genes (11). The activity of this HIF transcription regulator is itself governed by prolyl hydroxylases. In the presence of a sufficiently high concentration of molecular oxygen, these prolyl hydroxylases convert specific prolyl residues in the HIF-1α subunits into hydroxyproline. This allows capture by the von Hippel-Lindau tumour suppressor E_3 ligase complex, leading to ubiquitination and destruction, thus preventing HIF activity at higher oxygen concentrations. Below 1% O_2/1 kPa (10 000 ppm O_2), oxygenation is too small to induce degradation of the HIF-1α subunit, and HIF-1 accumulates instantaneously and influences specific promoter regions (12, 13). A second mechanism regulating HIF-1 activity is mediated by an asparaginyl hydroxylase, which modulates transactivations by HIF-1.

Downstream of HIF, carbonic anhydrase IX (CAIX) is an important example of a factor with a number of other functions. It is furthermore responsive to an upward shifted range of hypoxia (0.1–5% O_2/kPa or 1 000–50 000 ppm) and has a longer half-life.

Moderate hypoxia first results in a general translation inhibition, which later switches to a more specific, cap-dependent translation inhibition. Certain genes, including HIF-1 and VEGF, contain an internal ribosome entry site (IRES) allowing ribosomes to bind directly to the mRNA, thus bypassing the cap-dependent transcription repression (14).

In vitro stress proteins, e.g. HSP70 and HSP90, may become expressed, but may also appear as a response to reoxygenation (15) and function as a nuclear transfer factor by HIF-1.

The functional goal of these different cell activities can be seen as a restoration of the oxygen supply by HIF-1 mediated upregulation of the growth factor (VEGF), the platelet-derived endothelial cell growth factor vasodilating NO and erythropoietin production (16).

From 0.1% O_2 (1 000 ppm) down to 0.001% O_2 (10 ppm) is the range of severe hypoxia where cell survival is affected after a variable duration of hypoxia. 0.1% O_2 is about the oxygen concentration where aerobic glucolysis yields to ATP production by anaerobic glucolysis (2, 10). The low-energy output by anaerobic glucolysis is in normal tissue and during short periods of severe hypoxia partly countered by increased glucose uptake by the cells, but not necessarily so in tumour tissue (17).

Protein accumulation is greatly diminished, partly due to reduced overall protein synthesis as a result of phosphorylation of the eukaryotic initiation factor eIF2R and partly due to a slight upregulation of overall protein degradation (18). mRNA splicing may be altered at hypoxia (19). The inhibitor of HIF-1, iPAS, is a splicing variant of HIF-3.

At the low end of severe hypoxia, mRNA translation – the most energy-costly process in the cell – is much suppressed, as is ATP production. Respiration is reduced, and there is a limit as to how long cells can stay alive. However, there are two regulatory

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processes aiming at opposite goals: On the one hand, through cell cycle regulatory processes, cells are able to increase their probability of survival. On the other hand, through induction of apoptosis, damaged cells commit suicide, thereby protecting tissues from problems later on due to multiplication of damaged cells. At 0.05% O_2 (500 ppm O_2), cells are still cycling. However, from 0.05–0.5% O_2 (500–5 000 ppm), the ribonucleotide reductase activity is less than maximal owing to lack of molecular oxygen. This results in reduction in DNA synthesis and cell cycling.

In the range between 0.005 and 0.05% O_2 (50 and 500 ppm), cell division will continue until reaching the late G_1 phase (20).

The functional goal of many of the above adaptations can be seen as protection against hypoxia-induced cell death.

Below 0.001% O_2/1 Pa (10 ppm) we have the extreme hypoxia (anoxia), at which cell survival is a matter of hours or a few days, and no respiration takes place (2, 21).

We are talking about a state of oxygen deficiency which in vivo is known to exist in areas of solid tumours and infarct tissue (22). Cells introduced abruptly to this condition are arrested at certain restriction points. Cells in S phase stop DNA synthesis momentarily and are lethally damaged after a few hours, while cells in early G1 progress to a restriction point in G1 where they are arrested, but able to survive for many hours. Cells in G2 may complete cell division before they are arrested in the subsequent G1 with a similar ability to survive as G1 cells. Cells may die in the energy-requiring apoptosis or due to other mechanisms. Generally, cells in S phase will survive for less than 10 hours, while cells in G_1 may survive for up to 24–48 hours (21).

The cells' different responses to different oxygen concentrations offer new possibilities for *therapy*.

For tissue with moderate hypoxia, angiogenesis is a target in clinical anticancer trials, and at severe hypoxia bioreductive drugs have proved effective in tumour therapy. In the oxygen concentration range in between, it is to be expected that targeting HIF-1 and downstream processes for enhancement or inhibition will become of major importance in cardiovascular and solid cancer management (13).

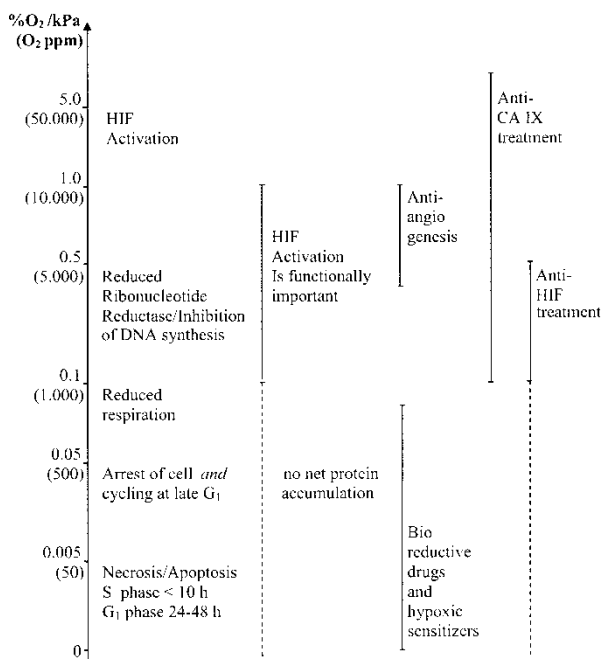

Fig. 1. Relationships between oxygen concentrations and important cell functions. 1% O_2 is roughly equal to 1 kPa and 1 kPa equals 10 000 ppm at sea level.

A first estimate of relationships between oxygen concentration ranges and important cell functions is given in Fig. 1.

We would like to stress that the present estimates of pericellular oxygen tension, especially in vitro, are hampered by a lack of measurements and use of electrodes measuring at a distance from the cells. Working out better and practical techniques for measuring oxygen is a pressing task.

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ADDENDUM

The SI unit for pressure is 1 Pascal (Pa) = 1 Newton per m^2, 1 atmosphere = 760 mm Hg = 1.013×10^5 Pa or approx. 100 kPa. The fraction of oxygen molecules in air is 21%, corresponding to 210 000 ppm (parts per million). Partial pressure of oxygen under 1 atmosphere = 21.2 kPa. Dissolved oxygen in water in contact with air under 1 atmosphere pressure, 21.2 kPa, is 220 μM (micromolar). The lower limit for oxygen partial pressure for full respiration at 37°C (measured with Ehrlich ascites cells) is 0.13 kPa, corresponding to 1.4 μM oxygen dissolved in the extracellular fluid (Ebbesen et al. 2000 (1)).