Look ma, no air

Until recently, it has been conventional thinking that mammals cannot live without oxygen. This has been challenged by studies demonstrating survival of mole rats without oxygen for extended periods of time. The ability of mammalian cells to survive in sub-atmospheric oxygen levels has been further substantiated by findings showing that although humans move through an atmosphere rich in oxygen (21%), our internal tissues experience oxygen levels that maximally are hypoxic (12% maximum oxygen level detected). In fact, numerous studies indicate that hypoxia is the normal state of oxygenation for the internal body environment. However, there are also extensive areas in the body that intermittently, or consistently totally lack oxygen; thus, these areas are anoxic/anaerobic. The most common body sites which are anoxic to severely hypoxic (less than about 3% oxygen level) include stem cell niches, mucosal surfaces, such as the gastrointestinal tract and vagina, as well as the centers of solid tumors. The roadblock to studying anoxic cell metabolism has been the inability to maintain cells without oxygen for longer than a couple of days. We developed a methodology that enabled mammalian cells to survive and replicate in the absence of oxygen using substitutes for oxygen to generate energy, i.e. anaerobic respiration. In our paper, we show that transformed and immortalized mammalian cells survive for at least 15 days without oxygen, with survival accompanied by a phenotypic change in cell appearance. Cells grown in the absence of oxygen are fundamentally different as compared to their aerobic (5% CO_2 in air) counterparts. When cultured anaerobically, by the third day, a shift occurs that results in two distinct cell morphologies comprised of dendritic-like attached cells and cells in suspension, which lack the ability to attach. Interestingly, the plasticity of cell phenotype in response to the presence of oxygen remains since anoxic Vero and HeLa cells in suspension (10 days anaerobic), when re-exposed to oxygen, revert to their aerobic monolayer morphology. In addition, there is production of reactive oxygen species (ROS) and variable expression of hypoxia inducible factor (HIF), a global gene regulator.

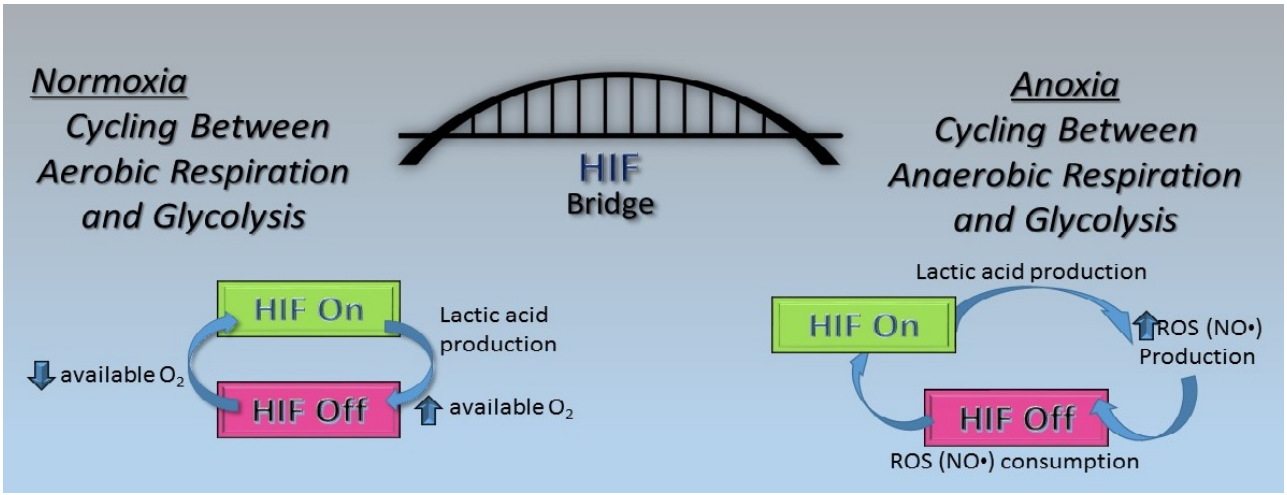

Fig. 1. Schematic diagram of the proposed link between aerobic (oxygen-based) respiration and anaerobic (non-glycolytic) respiration.

In hypoxic conditions, HIF cycles on and off depending on oxygen levels. We have found that when cultured anaerobically, cellular release of reactive oxygen species, like nitric oxide (NO) also affect HIF expression following the same pattern as that observed for hypoxic growth (Fig. 1). Functionally, our findings indicate that HIF expression, and the glycolysis induced, acts as a bridge between aerobic respiration, where oxygen acts as the terminal electron acceptor for the cytochrome oxidase system, and anaerobic respiration, in which a terminal electron acceptor other than oxygen is the terminal electron acceptor. This ability to undergo anaerobic respiration is likely a remnant of eukaryotic cell evolution, which began 2.7 billion years ago, half a billion years before the oceans became oxygenated. Thus, the mitochondrion of eukaryotic cells evolved at a time that required anaerobic respiration. These findings support our assertion that cell growth and metabolism under anaerobic conditions is divergent from aerobic growth. Furthermore, alterations in gene expression upon anaerobic growth may reveal new pathways and open new targets for cancer drug development, and enhance maintenance of organs *ex-vivo* for use in transplantation.

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Publication

A method for the long-term cultivation of mammalian cells in the absence of oxygen: Characterization of cell replication, hypoxia-inducible factor expression and reactive oxygen species production.
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