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Unregulated oxygen levels in mammalian cell culture changes cell physiology

As aerobic organisms we continuously utilize oxygen from the surrounding environment which ultimately participates in the formation of useful bioenergetic intermediates. Approximately 21% of the air we breathe is composed of oxygen, but the quantity of oxygen reaching the cellular level is quite variable, tissue-specific, and almost always lower. As the human respiratory and cardiovascular systems work together to deliver oxygen to tissues, the brain, skin, liver, and muscle all experience oxygen levels in the range of 2.5-6%. By the time oxygen enters the cell and reaches the mitochondrial respiratory chain – the site at which oxygen is utilized in energy production – those levels may be as low as 1.3%, even under basal conditions. While seemingly low, mitochondrial energy transduction to adenosine triphosphate is not usually oxygen-limited. This is because the main oxygen-consuming enzyme, cytochrome c oxidase, has an extremely high affinity for oxygen and is therefore oxygen-saturated and functioning maximally even when oxygen is present at seemingly low levels. Despite the low level of oxygen required by mitochondria to conduct metabolism, cell culture experiments are most commonly performed in normal atmosphere, which means that cells are bathed in ~18% oxygen. As it turns out, when left to grow in conditions where oxygen levels are higher than their normal physiological range, cells function differently. This is probably in part because cells transform some of the oxygen in their environment into reactive oxygen species (ROS).

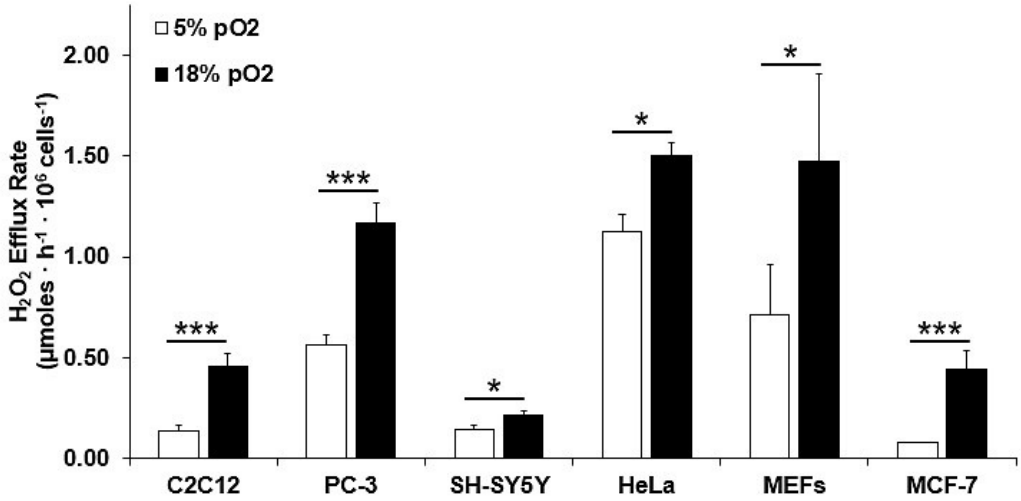

Fig. 1. H_2O_2 efflux rates produced by several commonly used cell lines. The data shows that more H_2O_2 is produced when cells are subjected to 18% (standard cell culture) versus 5% (physiological) oxygen. Bars represent the means ± SEM from ≥5 independent measurements. Asterisks represent differences between 5% and 18% O2. '*' = p-value < 0.05. '**' = p-value < 0.01. '***' = p-value < 0.001.

ROS are important molecules with various biological functions, but overproduction is associated with accelerated aging, neurodegenerative diseases, and cancer. ROS are produced from a variety of cellular sources. Superoxide is a progenitor ROS that often forms hydrogen peroxide (H_2O_2) and a wide range of other oxidative species. The mitochondrial respiratory chain doesn't function at 100% efficiency and some of the

oxygen in mitochondria is converted to superoxide radicals, which can in turn be rapidly converted into other ROS. NADPH oxidases, present in the cytosol and perhaps also mitochondria, similarly convert oxygen to superoxide or H_2O_2. A variety of other enzymes also produced ROS from oxygen. Although ROS are known to be important molecules in biological systems, little attention is usually paid to the relationship between oxygen and ROS production by animal cells.

We investigated ROS production by various cells lines commonly used in cell culture research when they were grown at a physiologically relevant oxygen level (5%) versus standard cell culture oxygen levels (18%). We did these experiments with muscle (C2C12 mouse myoblasts), skin (mouse embryonic fibroblasts; MEFs), and several human cancer cell lines (SH-SY5Y neuroblastoma, PC-3 prostate cancer, HeLa cervical cancer, and MCF-7 breast cancer). Using an enzyme-linked Amplex Red assay, we measured the amount of H_2O_2 emitted. In the presence of H_2O_2, Amplex Red reagent is converted to the fluorescent molecule Resorufin by Horseradish Peroxidase, whose fluorescence is directly proportional to the production of H_2O_2, allowing a measurement of H_2O_2 produced over time. The common trend we found was that all six cell lines we examined produced more H_2O_2 when cultured at standard oxygen versus physiological oxygen levels (Fig. 1).

We then set out to determine the source of H_2O_2 being produced under 5% and 18% oxygen conditions. Although mitochondria are generally asserted to be the main cellular sources of ROS, our experimental measurements suggested that this actually depends upon the oxygen level at which cells are cultured. Most of the increased H_2O_2 observed at high oxygen levels could be either completely abolished or ameliorated using a selective NADPH Oxidase isoforms 1 and 4 inhibitor, GKT137831 (Fig. 2). Interestingly, GKT137831 had little effect at low oxygen. These results suggest that NADPH oxidases are a major source of H_2O_2 under standard cell culture conditions, but perhaps not under more physiologically relevant oxygen conditions. Based on published data regarding NADPH Oxidase-4, which primarily produces H_2O_2 rather than superoxide and makes H_2O_2 three times as fast at 12% versus 3% oxygen, we believe that increased NADPH Oxidase-4 activity at 18% versus 5% oxygen is the main driver of the increase in H_2O_2.

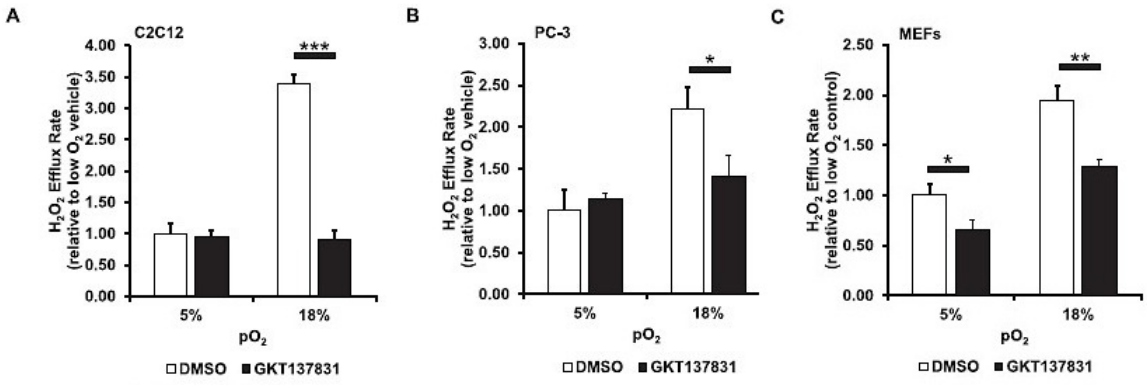

Fig. 2. NADPH oxidase inhibitor GKT137831 prevents the increase in cellular H_2O_2 production at higher O2 levels. (A) C2C12 cells. (B) PC-3 cells. C) MEFs. Bars represent the means ± SEM from ≥5 independent measurements. Asterisks represent differences between 5% and 18% O2. '*' = p-value < 0.05. '**' = p-value < 0.01. '***' = p-value < 0.001.

Taken together, our data indicate that oxygen levels within culture incubators have a major effect on rates of cellular H_2O_2 production/release. Since H_2O_2 affects a wide range of intracellular and extracellular redox signalling pathways, this could have important effects on cellular function. This highlights the importance of maintaining physiological oxygen levels during cell culture, especially for experiments directed at understanding how redox signalling pathways impinge upon cellular functions.

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Publication

Hydrogen peroxide production is affected by oxygen levels in mammalian cell culture.
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