

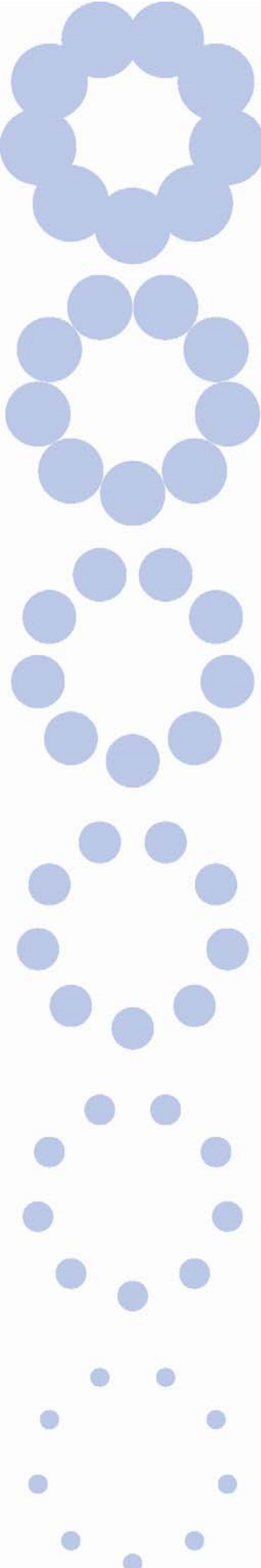


Hypoxic environment facilitates tumor cell research at Northwestern Feinberg School of Medicine in Chicago, USA

The ability to sense and respond to changes in oxygen is essential for the survival of multi-cellular organisms. Oxygen-sensing mechanisms within such organisms have been developed to maintain cell and tissue homeostasis, as well as to adapt to the chronic low-oxygen conditions found in diseases such as cancer. The pathways involved in oxygen sensing that are required for normal development and are deregulated in disease states are therefore of critical importance.

At Northwestern University, Feinberg School of Medicine (Chicago, IL, USA), Assistant Professor of Medicine Dr. Navdeep S. Chandel is conducting research on two fundamental questions regarding the biology of hypoxia: how do cells sense oxygen; how do cells die in the absence of oxygen?

Under low oxygen (hypoxic) conditions, tumor cells release factors such as vascular endothelial growth factor (VEGF) to promote the formation of new blood vessels - a process known as angiogenesis. The most well-characterized transcription factor regulating the hypoxia-induced angiogenesis commonly found in malignant tissue is hypoxia inducible factor 1 α (HIF-1 α). Although

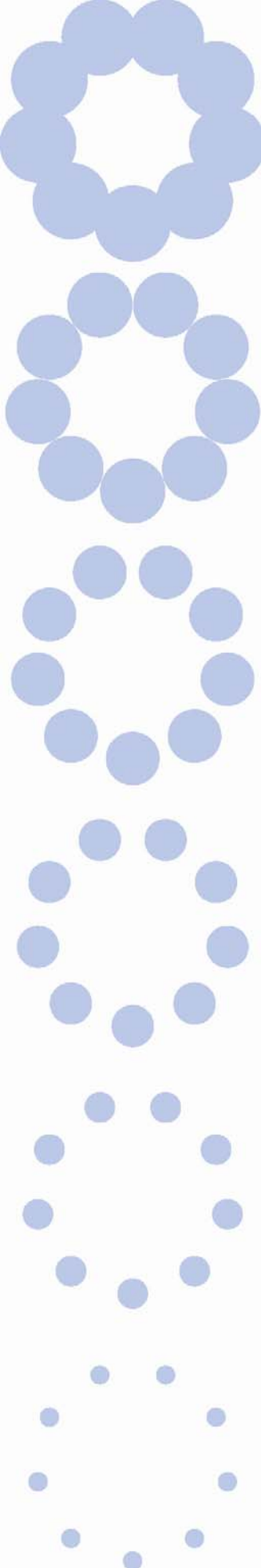


there has been much progress made in discovering factors that promote or suppress angiogenesis, the fundamental mechanism by which tumor cells or even normal cells detect the decrease in oxygen levels resulting in HIF-1 α activation and subsequent angiogenesis remain unknown.

Dr Chandel's work has recently investigated the role of mitochondria (responsible for cellular energy conversion) in regulating HIF-1 α protein stabilization under hypoxic (1-2% O₂) conditions.

To begin answering the first question of how cells sense oxygen, the school utilized Ruskinn Technology Ltd's Invivo₂ 200 hypoxia workstation, which can control oxygen concentrations, from normal atmospheric – down to 0.1% in 0.1% increments.

Stable and controllable hypoxic conditions (1.5% O₂, 93.5% N₂, and 5% CO₂) were achieved using the workstation's oxygen sensor to continuously monitor the oxygen tension. Once optimized, the experimental conditions were easy to maintain through the rapid interlock transfer process. Up to 10 samples can be transferred through the interlock from the bench in 15 seconds and most importantly, the sleeved port entry system allows bare hand access to a total capacity of 180 plates without altering the preset conditions of the workstation.

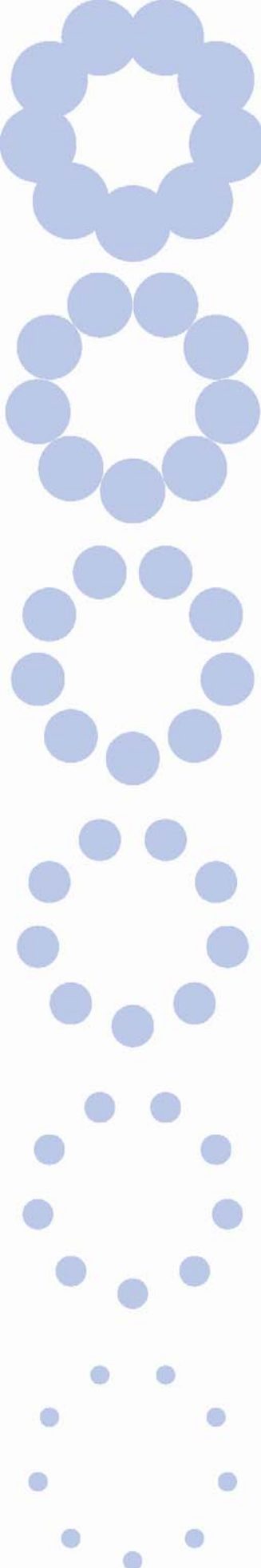


Although more traditionally used in the microbiology laboratory, the compact “BugBox” anaerobic workstation, also from Ruskin Technology Ltd, was used to attain anoxic conditions (0% O₂, 85% N₂, 10% H₂, and 5% CO₂). Dr. Chandel explained; “This allowed us to work at anaerobic conditions to begin addressing the second question of how cells die in the absence of oxygen”.

He continued: “Before purchasing the workstations, we previously used airtight jars with inflow and outflow ports for our hypoxia work. The inflow was hooked up to a tank with the appropriate gas mixture. These jars however only allowed a few plates and we wanted to be able to do multiple samples.”

The hypoxia workstation provides laboratories with an affordable step up in technology that can reproducibly provide stable environmental conditions. Most importantly for Dr. Chandel, “We can now change the oxygen level quickly and manipulate the 100 plates that we require at any one time. Also we can perform cellular extractions within the workstations i.e. under hypoxic conditions without oxidizing our samples.”

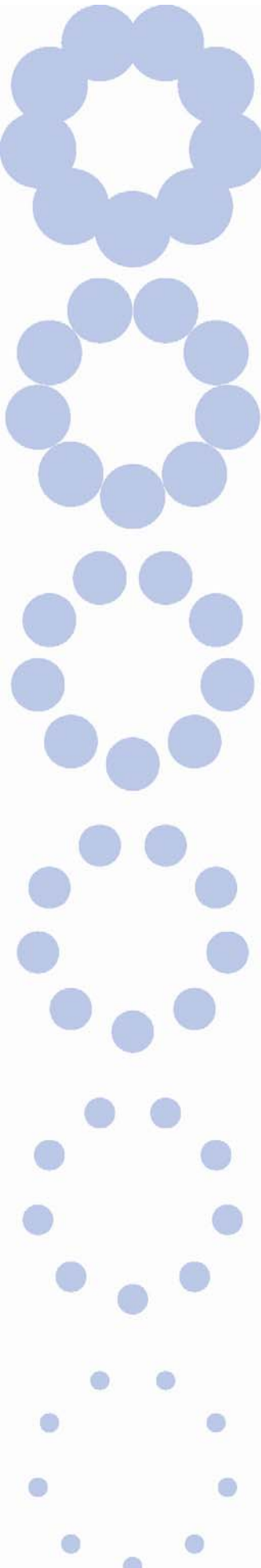
The molecular events regulating HIF-1 α protein stabilization during hypoxia are important for understanding the mechanisms of cellular oxygen sensing. Dr Chandel’s studies have found that the stabilization of HIF-1 α protein at hypoxic (1.5% O₂) but not anoxic (0% O₂) oxygen concentrations requires mitochondrial-dependent signaling.



The laboratory is now investigating the regulation of tumor angiogenesis and programmed cell death (apoptosis) in response to oxygen deprivation. The team is testing the hypothesis that mitochondria detect decreases in oxygen concentration and initiate the activation of HIF-1 α and the VEGF release from tumor cells under such conditions.

While tumor cells await angiogenesis to replenish deprived oxygen and nutrients, conditions develop where apoptosis may occur in response to a lack of oxygen and glucose. However certain mutations can occur that render tumor cells resistant to oxygen-deprivation induced apoptosis. The group is currently focusing on the mechanisms by which oxygen deprivation initiates apoptosis.

Dr Chandel feels it is important to find a way of standardizing the conditions used for hypoxia research. “The use of different technologies makes it difficult to define the oxygen concentrations used in experiments by other investigators”, he explained. “We feel if the hypoxia community would adopt a single technology to conduct their hypoxia/anoxia experiments then it would make it easier for us to evaluate each others’ data.”



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