Application Notes

A series of interviews conducted by HypOxygen, Don Whitley Scientific Limited's US distributor.

Prof. Biplab Dasgupta is at Cincinnati Children's Hospital, UC Department of Pediatrics, working on neuronal tumors.

a. *"The AMPK inhibitor Compound C is a potent AMPK-independent anti-glioma agent" (2014) Xiaona Liu, Rishi Raj Chhipa, Ichiro Nakano, and Biplab Dasgupta; Mol Cancer Ther. 2014 Mar; 13(3): 596–605.*
The therapeutic value of Compound C (dorsomorphin) for glioblastoma was evaluated in this study by Liu et al. The lab uses an Hypoxystation for cell culture based on the highly hypoxic nature of the tumor tissue. AMP Kinase, which functions as a cellular watchdog during metabolic crisis, is selectively inhibited by Compound C. Mechanistic studies revealed that the anti-proliferative effects of Compound C were AMPK-independent, and were significantly more pronounced in glioblastoma cells than in normal astrocytes.

b. *"The tumour suppressor LKB1 regulates myelination through mitochondrial metabolism" (2014) Shabnam Pooya, Xiaona Liu, V.B. Sameer Kumar, Jane Anderson, Fumiyasu Imai, Wujuan Zhang, Georgianne Ciraolo, Nancy Ratner, Kenneth D.R. Setchell, Yutaka Yoshida, Michael P. Jankowski and Biplab Dasgupta; Nature Communications 5, Article number: 4993*

Stem cell and other cellular differentiation processes are accompanied by a shift from glycolytic metabolism to oxidative, while de-differentiation in re-programming of pluripotent stem cells involves the transition back to a glycolytic metabolism. Many studies have shown that hypoxic culture increased cell survival, enhanced growth, increased cell yield, accelerated generation and self-renewal of stem cells, improved maintenance and increased longevity of stem cell pools. This study examines the role of the tumor suppressor Lkb-1 in differentiation of Schwann cells during myelination of peripheral axons. Deficient Lkb-1-mediated oxidative metabolism led to inadequate production of citrate and thus, impaired lipogenesis, and to peripheral neuropathy in mice.

The lab has been using an Hypoxystation for approximately 5 years now. HypOxygen asked how the lab uses the workstation for hypoxic cell culture.

What is the focus of the research at your lab?

Our lab is interested in the development of the central nervous system, particularly the brain in the light of cellular metabolism. While we have some understanding of the transcriptional control of brain development, it is largely unclear how the transcriptional program is connected to cellular metabolism

and how disruptions in these programs cause diseases of the central nervous system. We are interested in examining the biochemistry of cancer cells with a focus on a type of brain cancer called glioblastoma.

We study stem cells using mouse models, using AMP Kinase as a tool that does from the very obvious to very subtle things. It is a complex enzyme and we are trying to understand its role in the normal brain and under stressful conditions, because AMP Kinase is a stress sensor. We also study aging, whether AMPK is important for adult neurogenesis, as new neurons are born in the adult brain constantly, even though not all of them live. We use AMPK knockout mouse models, specifically to examine neural stem cells of the brain, where the AMPK might have a role in stress-induced neurogenesis of the adult brain. Studies show that exercise and certain diets induce cognitive power, and we'd like to know if AMP Kinase is involved in that process.

A major aspect of our research is definitely the role of AMP Kinase in brain tumors. AMPK is actually a strange enzyme that is part of a tumor suppressor pathway called LKB1-AMPK pathway, and while it has been known for a long time that AMPK is probably a tumor suppressor gene, there are no AMPK-inactivating mutations known in human cancers. Tumor suppressor genes are mutated or lost in human cancers, p53 and PTEN are classic tumor suppressors, and in fact LKB1, which regulates AMPK, is lost in a large portion of lung cancers. However, we do not see any mutational loss of AMPK. On the contrary, there are some tumors that actually show higher expression of the AMPK protein, so that is really a conundrum in the field, that AMPK is downstream of this tumor suppressor where the suppressor is lost, but AMPK is not. It turns out that AMPK function is context-dependent: in certain cancers it can have a pro-growth role, while it can inhibit growth in other cancers, all depending on the cancer type and the stage. Recent research shows that AMPK is a tumor suppressor in mouse B cell lymphoma, but at the same time, other groups have shown, mostly through in vitro studies more than mouse models, that AMPK does the opposite. For example, it likely functions as an oncogene in prostate cancer. We study glioblastoma, and we are trying to understand whether AMPK is a pro-tumor or an anti-tumor gene in glioblastoma, which is the deadliest form of brain cancer. Solid tumors like glioblastoma are highly hypoxic, they have very low oxygen concentrations compared to the rest of the brain, and it is interesting that such a hypoxic area should be conducive to the growth of certain cells. The hypoxic niche is likely the home for radiation resistant glioma stem-like cells that are the likely cause for relapse. Research shows that tumor hypoxia is actually the main deterrent to complete eradication of cancer cells by radiation. Hypoxia blocks the effect of radiation, but the pathways are not very well-known. That's why we use the hypoxia workstation, to understand certain pathways.

Can you describe the course of a typical assay with the brain cancer cells?

We use primary cells from patients, because we have found that glioblastoma cell lines that have been in culture for decades don't produce consistent results. Many of these lines have lost the ability to form tumors when they are transplanted into the mouse brain, and that is very worrisome. They have become totally different, the culturing has exacerbated their already unstable genome. We cannot use those cell lines. We get the cells from the patients and assess the genetic signature, because glioblastomas can be classified into 2 main types, proneural and mesenchymal. We have 20-30 of these primary tumor cell lines now, and we characterize their metabolic pathways, are they more glycolytic or

oxidative, how much of the pentose phosphate pathway do they use, how much glutamine do they require, and so on. Then we create a whole bunch of knock-downs and knock-outs, which we expose to different nutrient and oxygen conditions, to ascertain under what conditions optimal tumor growth occurs. The goal might be to assess under what conditions gene silencing becomes most effective. We grow the cells in the hypoxia workstation and we look at the hypoxia response, for example we examine one of the very important hypoxia-driven proteins called HIF-1α. Our research examines whether and to what degree there are interactions between HIF-1α and AMP Kinase, how HIF-1α is stabilized and what it does in these cells from different brain cancers.

During the culture process, what are typical steps the cultures require, and how many times total would you say are you going in and out for a typical cell culture?

These cells can stay in culture for an indefinite amount of time, as they are primary brain tumor cells. The problem with the primary cultures is that more than half of them won't establish in culture from the patient tissue, but those that do proliferate very aggressively in the Hypoxystation. It takes only about 10,000 cells to form a tumor and that kills the mouse in 2-4 weeks. Most researchers don't have access to an hypoxia workstation, so the ambient oxygen most people use is absolutely not normal. Our glioblastoma cells grow very well in the low oxygen as we try to mimic their physiological situation by changing the oxygen level to that seen by an aggressively growing tumor. Established cell lines, however, may simply die in hypoxia, because they are adapted to the non-physiological ambient oxygen levels.

Our typical experiments last for 3-5 days and we would always have our cells in the Hypoxystation. We isolate RNA inside the chamber, and we do protein lysis inside the chamber: we do not expose the cells to ambient oxygen before we extract RNA or proteins. If any sort of oxygen response is expected in our experiments, we incubate within the workstation.

How would you rate the stability of the oxygen environment?

We have had the Hypoxystation for 3 years and the oxygen level stays very stable.

You study a number of mice models for the AMP Kinase, did you have them in the workstation? Do you anticipate the need to have animals in the workstation?

We don't have mice in the workstation, because as the tumors grow, they automatically become hypoxic, they don't need that external parameter.

See more of what Biplab Dasgupta is doing here:
http://www.cincinnatichildrens.org/research/divisions/o/oncology/labs/dasgupta/default/