Part I. Introduction

A transcription factor is a protein that binds to specific DNA sequences, thereby controlling the rate of transcription of genetic information from DNA to messenger RNA. DNA binding proteins known as HIFs, short for Hypoxia Inducible Factors.

In vitro, the oxygen-regulated subunits HIF-1 and 2 are expressed in inverse relationship to oxygen tensions in every cell line investigated to date. HIFs are regulated at the protein level by oxygen-dependent enzymes and, hence, allow for tissue hypoxia detection. The cellular adaptation to hypoxia, as a result of HIFs sustains the growth of rapidly proliferating tissues such as developing embryos and solid tumors. Many of the cellular responses to a hypoxic environment are orchestrated by a group of these transcription factors, which induce the expression of genes that mediate a metabolic rewiring of the hypoxic cell, induce formation of new blood vessels around the hypoxic tissue, and can promote cell survival in this scenario, conclusively, HIFs are essential for embryonic development, but are also exploited by cancer cells during the progression of many solid tumors. The implications being that the HIFs have their own respective medicinal and research applications, from embryonic to angiogenesis, to cancer. The molecular mechanisms that HIFs follow allows a better understanding of their regulation of the transcriptional apparatus that enables the cellular and organismal response to hypoxia. Under conditions of normal oxygen tension, the alpha subunits of the HIF’s are hydroxylated at key proline and asparagine residues, in turn inhibiting their function. Upon hypoxia, the HIF alpha subunits are stabilized and accumulate in the nucleus, where they dimerize with the HIF1 beta subunits (HIF1B). HIF1B is stably expressed and is also an obligate partner for the aryl hydrocarbon receptor. The dimerization of HIF alpha subunits and the HIF1B allows them to bind to DNA and stimulate the transcription of their target genes. This allows the coordinate activation of genes essential in the adaptive response to hypoxia including pathways that decrease the cellular demand for O2 and increase O2 delivery and tissue re-oxygenation. The interplay between these two HIF alpha sub units is an interesting dynamic, as it could be possible that they do not interfere with each other at all, or may they are more dependent than anticipated. Even though it is known that they are two separation entities, it is still possible that they may pose some sort of an effect on the other as HIF2A shares high sequence homology with HIF1A and functions in a similar manner. HIF2A also dimerizes with HIF1B upon hypoxic induction. Different from HIF1A, HIF2A stimulates the expression of its own distinct set of target genes. What effect does the suppression of HIF1A in a hypoxic environment have on the expression of HIF2A?

Part II. The Experiment

The overall strategy that will be used will first begin with using renal tissue of rats as a template for the experiment. The reason for using rats is that they are easy to come by. Rosenber et al, 2002 did mention any other specific reason for using rats. For the collection of renal tissue, the animals received intraperitoneal injections of sodium pentobarbital to be used as a sedative. In experiments with exposure to low ambient oxygen level, animals were returned to the chamber after injection and the chamber was flushed with the respective gas mixture to keep the proper levels of oxygen. (Rosenberg et al. 2002) Once the control kidneys and the hypoxic kidneys are seperated, the HIFs will be measured through high amplification immunohistochemical analyses. This is the ideal method to use to measure HIFs because previous studies in rats revealed the need for standardized fixation, special target retrieval, and high-amplification technique to obtain reliable results (Weisner et al 2002, Rosenberg et al. 2008). The control kidkeys aside, the rest of the hypoxic kidneys will be subject to the protein hypoxia-associated factor (HAF). HAF also regulates the stability of alpha subunits via ubiquitination and proteasomal degradation; however, unlike other regulators such as phosphokinases, they do so independently of oxygen tension (Liu et al. 2007). HAF ubiquitinates HIF1A, but not HIF2A, targeting it for destruction regardless of oxygen tension, making it an ideal protein to use for the removal of HIF1a, leaving only the HIF2a. (Koh et al. 2008). This will effectively eliminate the the HIF1a, leaving only the HIF2a, which can then be measured to see what has occurred. To conduct the immunohistochemical analysis paraffin sections of 4m in length were dewaxed in xylene, rehydrated in a series of ethanol washes, and placed in distilled water before staining procedures. Slides were coated with 3-aminopropyl-tri-ethoxysylane. For detection of HIF isoforms, monoclonal mouse anti-human HIF-1 antibody (Rosenberg et al. 2002) and polyclonal rabbit anti-mouse HIF-2 antibodies were used. Specific staining of each HIF isoform was confirmed in immunoblots by using in vitro transcribed and translated mouse HIF-1 and HIF-2. The signals will be analyzed with a Leica DMRB microscope using differential interference contrast. Photographs were digitally recorded by means of a Visitron system as they were used successfully previous experiments (Warnecke et al. 2003). The kidneys will be measured daily for a period of 3 days to ensure consistent results. The resting kidneys will stay in hypoxic chambers at all times, except for when they will be measured.

Part III. Discussion

There is a sufficient amount of evidence to support the view that the HIFs’ are ubiquitous, instantaneously up-regulated upon hypoxia, and short-lived when sufficient oxygen is reestablished. That being said, however, there is not much evidence as to how the absence of one HIF alpha sub unit affects the other. This is usually because the experiments that revolve around measuring HIFs either only measure one type of HIF at a time, or the ones that measure both are doing them concurrently. To clarify, this experiment is comparing both HIFs when they are present at the same time, and then comparing those results to when one is suppressed, in this case HIF1a, and the other isn’t. In addition to HIF2A having more restricted expression across tissues, there is evidence supporting the notion that there is a time delay in play that affects when HIF1a and HIF2a respond. Under conditions of acute hypoxia (i.e. < 24 hours’ stimulation), transactivation of target genes occurs primarily by virtue of HIF1A, whereas following longer periods HIF2A begins to exert more of an influence (Koh et al., 2011, Koh and Powis, 2012). Thus, a potential result of suppression HIF1a could be that there is no temporal delay for the incidence of HIF2a. Additionally, HIF1A regulates the activation of glycolytic genes, thereby allowing cells to survive under conditions of decreased oxygen by switching the metabolic scheme from one of oxidative phosphorylation to anaerobic glycolysis. Several studies demonstrate that HIF2A plays no role in glycolytic gene regulation, but rather induces the expression of genes involved in pluripotent stem cell maintenance and angiogenesis (Hu et al., 2003, Takeda et al., 2004, Koh et al., 2011). If this is the case, then it can be possible that the cell takes additional adaptions from HIF2a, to survive without the presence of the HIF1a. A potential pitfall this experiment could have is if the renal cells die too quickly in a hypoxic environment, if the HIF1a’s adaptions are not in place. This is only an issue if HIF2a doesn’t react to the lack of HIF1a. A potential remedy to this pitfall would be to use tissue that can survive longer in hypoxic environments. The limitations of this experiment are that it does not encompass the affects of the suppression of HIF2a on HIF1a, and also this experiment has no mention of the third HIF factor found in mammalian cells, HIF3a. If this were a larger experiment, then all permutations of suppression and affect could have been tested from the three distinct HIF factors.

Part IV. Citations

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