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Hypoxic behavior in cells under controlled microfluidic environment

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ABSTRACT

Background: Depleted oxygen levels, known as hypoxia, causes considerable changes in the cellular metabolism. Hypoxia-inducible factors (HIF) act as the major protagonist in orchestrating manifold hypoxic responses by escaping cellular degradation mechanisms. These complex and dynamic intracellular responses are significantly dependent on the extracellular environment. In this study, we present a detailed model of a hypoxic cellular microenvironment in a microfluidic setting involving HIF hydroxylation.
Methods: We have modeled the induction of hypoxia in a microfluidic chip by an unsteady permeation of oxygen from the microchannel through a porous polydimethylsiloxane channel wall. Extracellular and intracellular interactions were modeled with two different mathematical descriptions. Intracellular space is directly coupled to the extracellular environment through uptake and consumption of oxygen and ascorbate similar to cells *in vivo*.
Results: Our results indicate a sharp switch in HIF hydroxylation behavior with changing prolyl hydroxylase levels from 0.1 to 4.0 μM. Furthermore, we studied the effects of extracellular ascorbate concentration, using a new model, to predict its accumulation inside the cell over a relevant physiological range. In different hypoxic conditions, the cellular environment showed a significant dependence on oxygen levels in resulting intracellular response.
Conclusions: Change in hydroxylation behavior and nutrient supplementation can have significant potential in designing novel therapeutic interventions in cancer and ischemia/reperfusion injuries.
General significance: The hybrid mathematical model can effectively predict intracellular behavior due to external influences providing valuable directions in designing future experiments.

1. Introduction

Rapid tumor growth can result in localized zones in the tumor microenvironment where cells have far less access to nutrients. It has often been observed that in these zones cells get very little oxygen due to the inadequate or defective vasculature, a condition known as hypoxia [1]. The irregularities in functional behavior as well as structure of the blood vessels in solid tumors have been identified as one of the main reasons for oxygen deficiency in the tissue [2]. Hypoxia is also known to be induced in heart and brain cells through ischemia [3]. In some instances, these zones might have no access to oxygen supply at all (a condition known as anoxia) which could lead to cell apoptosis and necrosis [4]. Although it is known that healthy cells can be severely damaged in hypoxia, cancer cells show significant resistance in hypoxic and anoxic conditions [5]. Moreover, hypoxic cancer cells have shown decreased susceptibility to radiotherapy and chemotherapy [6]. It is, therefore, a topic of interest from the therapeutic point of view to study the cell behavior in hypoxia.

Hypoxia-inducible factors (HIF) are a family of transcription factors that are highly active in cells under conditions of low levels of oxygen [7]. In normoxic conditions, isoforms of the prolyl hydroxylase (PHD) domains in the cell cytoplasm bind with HIF and act as the HIF regulator [8] (see Fig. 1). This regulation, however, is dependent on the kinetic activation (or catalysis) of PHD by a number of other nutrients [9]. In hypoxic and anoxic conditions, the lack of oxygen impedes the hydroxylation of HIF. This causes an accumulation of HIF1α, a prominent HIF isoform, in the cytoplasm which shuttles into the nucleus [10] and binds with HIF1β to form a dimer complex (HIF_d) [11]. This HIF dimer transcriptionally activates large number genes at the hypoxia response element (HRE) sites. Significant target genes include those controlling erythropoiesis and iron metabolism [12]; influencing proliferation like insulin growth factors (IGF) and transforming growth factors (TGF) [13]; regulating vascular tone and angiogenesis such as vascular endothelial growth factor (VEGF) [14]; controlling energy metabolism like aldolase, enolase, pyruvate kinase and glucose transporters [15]. All these factors have made the cellular dynamics of HIF a topic of prime interest in the study of ischemia and tumor growth [16].

Although it might have been ideal to study the evolution of HIF-mediated cellular response in human cells *in vivo*, it is still not

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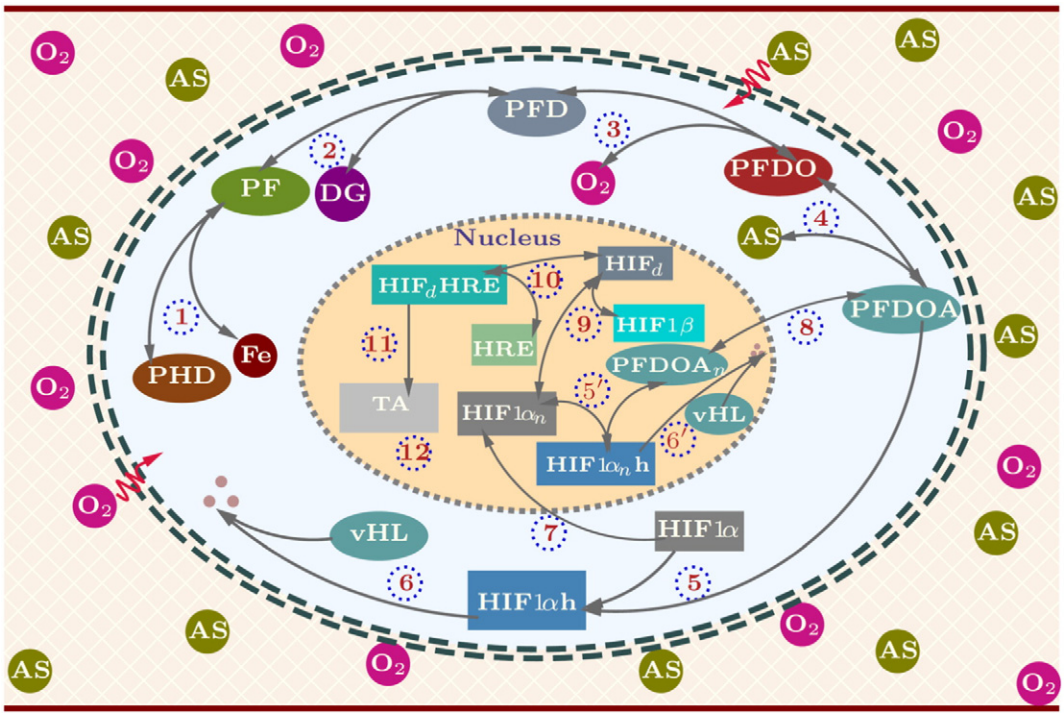

Fig. 1. Schematic of the normoxic and hypoxic reaction pathways. Under normoxic conditions prolyl hydroxylase domains (PHD) form complex with iron (Fe), 2-oxoglutarate (DG), oxygen (O_2) and ascorbate (AS) (1–2–3–4), and hydroxylates the hypoxia-inducible factors (HIF1α) (5), which later gets degraded by ubiquitination reaction after being tagged by von Hippel-Lindau (vHL) proteins (6). Under oxygen tension, more HIF1α is available to pass inside the nucleus (7), where aside from being engaged by PHD complex (5′-6′), they undergo dimerization with HIF1β (9) which is primarily available inside the nucleus. The dimer (HIFd) then reacts with hypoxia response elements (HRE), and eventually serves as the key element in transcriptionally activating (TA) a wide array of genes responsible for angiogenesis, tumor progression, and survival. Shuttling of PHD in and out of the nucleus (8) has been assumed to occur after catalytic activations (1–2–3–4) for simplicity, and uptake of extracellular nutrients (O_2 and AS) has been considered for proper representation of the biophysical activities.

technologically or logistically feasible. A large number of *in vivo* mouse xenograft models [17] and *in vitro* studies using different cell lines have clearly shown the accumulation of HIF1α in the cell and the associated transcriptional activities [15]. Microfluidic cell culture devices have been used in recent years as *in vitro* platforms where it is possible to precisely maintain viable microenvironments (both temporally and spatially) for studying cellular pathways [18]. For instance, microfluidic devices have been used to study cancer cell growth, cell cycle, apoptosis, and other mechanisms [19]. These low-cost devices can particularly be used to study multiple cell lines under exactly same nutritional and pathological conditions providing better integration of operations and an efficient way to study the heterogeneity of cell populations [20,21]. Moreover, these devices can be used for anticancer drug testing and to study the cellular response over desired time span under easily reproducible conditions, which are not possible in traditional *in vitro* experiments [22]. More importantly, the microenvironment in these microfluidic cell culture devices can be used to closely mimic many *in vivo* phenomena like the transition to invasive phenotype of the cells in the tumor microenvironment [23,24].

Study of cellular response to oxygen starvation and nutrient supplementation in the microfluidic environment necessitates simultaneous spatial and temporal measurements of a large number of species like different forms of prolyl hydroxylase domains, hypoxia-inducible factors, ascorbate, oxygen, iron etc., which presents a significant challenge in the currently available optical measurement techniques. Moreover, microenvironment-dependent extracellular to intracellular and cytosolic to nuclear shuttling of the nutrients play a crucial role in determining the outcome of the cellular dynamics. However, it is extremely difficult to measure concentrations of the same species in different cellular compartments like the nucleus, mitochondria and the cytoplasm *in vitro*. Mathematical models are of significant benefit in this regard, where it is possible to investigate selected pathways in greater detail than it is possible with experimental methods. Coupled with experimental studies, mathematical models of the cellular microenvironment can provide meaningful insight into therapeutic studies. More importantly, it can venture beyond the logistical limitations of experimental assays and provide directions for specific experimental studies, thus saving resources and time.

A number of mathematical models have been presented with different levels of detail for the intracellular reactions in hypoxic condition. Some of these models investigated pathways involving oxygen sensing mechanism by HIF1α and the roles of different intracellular components on HIF1α hydroxylation [25], while others focused into cytosolic to nuclear shuttling of nutrients and HIF1α at different stages of the HIF1α hydroxylation as well as post-hypoxic activities in a single cell model [26]. All these models, however, consider only the intracellular dynamics and for the most part, treat the species inside as a well-mixed system, resulting in a set of ordinary differential equations representing the cellular dynamics. However, it is well established that the microenvironment appreciably influences cellular metabolism especially when the supply of nutrients are being impeded externally [27]. It is also known that cellular uptake and consumption of nutrients like oxygen, ascorbate and iron from the extracellular environment are continuous processes, which cannot be properly represented only with an intracellular mathematical model. Additionally, the available cell-based intracellular models are not adequate to provide a satisfactory description of the microfluidic cell culture device, where nutrients are supplied in the extracellular domain, and the hypoxic environment is also maintained using extracellular control.

In this study, we have presented a hybrid model to study the transport and evolution of different species in both extracellular and intracellular spaces. Changes in the upstream conditions, such as nutrient supplementation and oxygen extraction in the extracellular domain, have been incorporated to represent the actual microfluidic