Hypercapnia-inducible factor: a hypothesis

Khachik K. Muradian¹, Vadim E. Fraifeld^{1,2}

¹ D. F. Chebotarev Institute of Gerontology NAMS of Ukraine, Kyiv, Ukraine

² The Shraga Segal Department of Microbiology, Immunology and Genetics, Center for Multidisciplinary Research on Aging, Ben-Gurion University of the Negev, POB 653, Beer-Sheva 8410501, Israel

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Correspondence: muradian1944@gmail.com OR vadim.fraifeld@gmail.com

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Abstract. Cells and tissues sense and respond to hypercapnia by global activation or down-regulation of hundreds of genes and switching on/off a number of signaling and metabolic pathways. We hypothesize for the first time that such complex rearrangements are hardly possible without subtle guidance by a specific master regulator which we suggest to name hypercapnia-inducible factor (HcIF). Whether there are structural and functional similarities between HcIF and HIF remain to be elucidated. However, there are reasons to believe that, as master genes, HcIF and HIF can cooperate or compete depending on the situation. Only further research will warrant existence of HcIF as a molecular master regulator of the response to hypercapnia.

Key words: hypercapnia, hypoxia, gene expression, aging

Introduction

Oxygen (O₂) and carbon dioxide (CO₂) are correspondingly the substrate and product of the energy-generating oxidative reactions (burning) and their biological equivalent – mitochondrial oxidative phosphorylation. It is well established that cells and tissues can sense and respond to various gases and primarily to O₂ and CO₂ by switching on/off the corresponding genes and signaling or metabolic pathways [1-5]. In fact, O₂ and CO₂ are the most important gases for a living organism. Even short term overly deviation of their concentrations from normal values in the air or partial pressure in tissues (P_{O₂} and P_{CO₂}) could have dramatic consequences. Of particular interest are the often situations of decreased P_{O₂} (hypoxia) and reciprocally increased P_{CO₂} (hypercapnia). The resulting hypoxic-hypercapnic environment (HHE) is typical for species with extreme longevity, as well as for aging and aging-associated pathology, especially for stroke, heart attack and neoplasia. It is also noteworthy that HHE is practically inevitable in embryonic development of most, if not all, animal species, so that all, or nearly all, living beings start their existence in HHE [6].

Crosstalk between O₂ and CO₂ has deep evolutionary roots. Biological life originated around four billion years ago when the Hadean atmosphere was dominated by CO₂ and there were only trace amounts of O₂ in the earth atmosphere [7-9]. The time has passed and O₂ and CO₂ in the current atmosphere returned almost to the same kind of values, though this time O₂ was the superior. Its content in atmosphere increased up to 21% whereas CO₂ content dropped to 0.04%. However, despite the more than 500-fold difference in the atmosphere, P_{O₂} and P_{CO₂} in most animal tissues display close values (5-7%).

Hypoxia-inducible factor (HIF)

The atmospheric changes in O₂ and CO₂ contributed to the development of regulatory systems which obviously impacted evolutionary trajectory and current status of biological objects. Nowadays, it is recognized that hypoxia triggers massive metabolic and gene expression rearrangements orchestrated by the master gene of hypoxia. It was discovered in 1995 and named hypoxia-inducible factor (HIF) by Gregg Semenza [10, 11]. The 2019 Nobel Prize in Physiology or Medicine was granted jointly to William G. Kaelin Jr., Sir Peter J. Ratcliffe and Gregg L. Semenza “for their discoveries of how cells sense and adapt to oxygen availability”. Further research revealed that HIF represents a family of transcriptional activators which regulate the oxygen homeostat in all types of multicellular organisms by coordinated switching on/off of hundreds of genes [11]. HIF-1 is the best-known member of the HIF family and consists of two subunits, HIF-1 α and HIF-1 β . Under normal conditions, HIF-1 β is present in excess, so that only the level of HIF-1 α determines functional activity of HIF-1. Both subunits contain helix-loop-helix domains which allow them to join to the HIF-binding DNA segments of subordinate genes and change the level of their transcription [10, 12]. In response to hypoxia, the transcription rate of hundreds of genes increases in the HIF-dependent manner. At the same time, the transcription rate of a comparable number of other genes decreases, also by the HIF-dependent mechanism, but without direct binding of HIF-1 to the promoters of respective genes. Under hypoxic conditions, HIF-1 switches metabolism from oxidative phosphorylation to glycolysis by inhibiting the conversion of pyruvate to acetyl coenzyme A and enhancing the conversion of pyruvate to lactate. Additionally, population of mitochondria is reduced due to activation of mitochondrial autophagy [11]. Recent findings highlighted intriguing role of HIF-1 in tissue regeneration and rejuvenation of aged skin and heart cells [13, 14], and in hypoxia-induced life span extension in mice [15].

Hypercapnia-inducible factor (HcIF)

Hypercapnia also induces global rearrangement of gene expression comparable with that in hypoxia [1, 3]. For example, exposure of model organism *C. elegans* to elevated levels of CO₂ for 3 days caused more than a twofold increase in expression of 771 genes and more than a two-fold decrease in expression of 657 genes (around 7% of all genes). Remarkably, maintenance of worms in such environment caused their life span extension by some 50% [16, 17]. In our experiments, the life-long maintenance of fruit flies in hypercapnic atmosphere also decreased the metabolic rate and extended their life span [18, 19].

It is reasonable assuming that coordinated switching on/off hundreds of genes and regulation of respective signaling and metabolic pathways in hypercapnia, as in case of hypoxia, is hardly possible without master gene(s). We therefore hypothesize the existence of hypercapnia-inducible factor (HcIF), in analogy with HIF. At current stage of knowledge, it is difficult to imagine how far the analogy between HIF and HcIF could be extended. However, it is almost for sure that HIF and HcIF have different targets and, as master regulators, can compete or cooperate with each other depending on the real situation. In favor of HIF and HcIF competing were the results of experiments on mice or cell cultures showing that hypercapnia suppresses the gene expression and decreases the stability of the HIF-1 α protein by activating the lysosomal degradation of the HIF-1 α protein [20]. In our experiments with mice kept for 30 days in self-produced HHE, we also observed a decreased expression of HIF-1 α during the entire period of HHE (paper in preparation). As for the examples of possible cooperation, it was shown that the healing process of the damaged endothelial layer was suppressed under hypoxia, while under the simultaneous action of hypoxia and hypercapnia wound healing was activated by stimulating cell proliferation [21]. We also observed accelerated skin wound healing of mice exposed to HHE, despite the decreased metabolic rate and body temperature [22]. The metabolism suppressing effects of HHE could be an important and evolutionary well-preserved phenomenon mediated by coordinated gene expression. For instance, maintenance of grain consuming insect cowpea bruchids in the air-tight containers resulted in gradual decrease of O₂ and accumulation of CO₂ with proportional decline of feeding activity which virtually ceased at around 3-6% of O₂ and 15-18% of CO₂. Profiling of transcriptomic responses to hypoxia/hypercapnia (3% O₂ and 17% CO₂ for 24 h) by DNA microarray and qPCR analyses revealed 1046 cDNAs around half of which did not show homology with the

known hypoxia-responsive genes and possibly could be related to hypercapnia [23]. It is reasonable to suggest that there might be certain number of specific and identical (overlapping) genes and metabolic pathways controlled by HcIF and HIF. However, much remains to be elucidated how hypercapnia activates genes and signaling pathways and to what extent they are conserved across species. A large-scale transcriptomic study of hypercapnia-induced genomic responses in mammals (mice and humans) and invertebrates (*C. elegans* and *D. melanogaster*) revealed evolutionarily conserved role of high CO₂ in regulating Wnt pathway genes [3]. Of particular interest could be if the number of hypercapnia-subordinated genes would change in aging and correlate with longevity, as was observed for HIF. Using available datasets, we are currently checking these possibilities *in silico*.

Conclusions

A cell and tissue response to hypoxia is obviously associated with activation or down-regulation of hundreds of genes and switching on/off a number of signaling and metabolic pathways. Regulation of such complex processes in hypoxia is conducted by a master gene known as HIF. Complexity of a cell or tissue responses to hypercapnia is obviously close to that of hypoxia and is also associated with activation or repression of hundreds of genes and associated signaling and metabolic pathways. We hypothesize for the first time that such complex rearrangements are hardly possible without specific master regulator(s) which we suggest to name hypercapnia-inducible factor, HcIF. At this moment little is known if there are structural and functional similarities between HcIF and HIF. However, there are reasons to believe that, as master genes, HIF and HcIF may cooperate or compete depending on the situation. Further research will approve or deny the existence of HcIF as a molecular carrier of master regulator of biological effects of hypercapnia.

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Information about Authors:

Khachik K. Muradian – DSc (Biology), Chief Researcher of the Department of Aging Biology and Experimental Life Extension of the D. F. Chebotarev Institute of Gerontology NAMS of Ukraine; <https://orcid.org/0000-0003-2515-6952>

Vadim E. Fraifeld – MD, PhD (Medicine), Chief Researcher of the Department of Aging Biology and Experimental Life Extension of the D. F. Chebotarev Institute of Gerontology NAMS of Ukraine, and Professor of the Shraga Segal Department of Microbiology, Immunology and Genetics of the Ben-Gurion University of the Negev; <https://orcid.org/0000-0002-4525-0535>

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