***Science* Global Home Page/Journal Page Title and Rotator Title**: Differential transcription for O_2 sensitivity

One-Sentence Summary and Rotator Teaser: HIF-2α and COX4I2 are necessary for carotid body oxygen-sensing (Moreno-Domínguez *et al.*, in 21 January 2020 issue).

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Genetic basis of oxygen sensing in the carotid body: HIF-2α and an isoform switch in cytochrome *c* oxidase subunit 4

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Abstract

The mechanistic basis of the marked oxygen-sensitivity of glomus cells in the carotid body has long puzzled physiologists. In this issue of *Science Signaling*, Moreno-Dominguez *et al.* show the critical importance of high levels of hypoxia-inducible factor, HIF-2α/EPAS1, and the nuclear-encoded mitochondrial cytochrome *c* oxidase subunit, COX4I2, in glomus cell sensitivity to hypoxia.

Understanding the control of breathing by the carotid body (CB) has intrigued physiologists ever since its chemosensory functions were proposed by De Castro and demonstrated by Heymans more than 80 years ago. These findings, together with subsequent work, established that the CB could be excited by changes in oxygen and carbon dioxide concentrations in the blood, as well as by inhibitors of mitochondrial respiration. These properties and the rapid time-scale of the CB neurosecretory responses distinguished the oxygen-sensing process from another paradigmatic response to reduced blood oxygen: transcription of the erythropoietin gene. Studies of erythropoietin led to the discovery of hypoxia-inducible factors (HIFs), which transduce a wide range of adaptive transcriptional responses in hypoxic cells. In the HIF system, the oxygen-sensitive signal is generated by a group of 2-oxoglutarate-dependent dioxygenases that catalyze the post-translational hydroxylation of HIFs. These enzymes are

inhibited by hypoxia but not by mitochondrial electron chain inhibitors, consistent with early observations on production of erythropoietin, which is encoded by a HIF target gene. However, despite intense investigation, the mechanisms of oxygen-sensing underlying the rapid neurosecretory responses of the CB to hypoxia have remained elusive. Nevertheless, early observations on mitochondrial inhibitor activity immediately suggested the 'metabolic hypothesis' in which reduced oxygen availability is sensed through an effect on mitochondrial respiration. In this issue of *Science Signaling*, Moreno-Dominguez *et al.*[1] provide intriguing new insights into this process.

An early concern with the metabolic hypothesis was that the oxygen affinity of mitochondrial complex IV (MCIV)/cytochrome *c* oxidase (the terminal enzyme in the respiratory chain that catalyzes the transfer of electrons from cytochrome *c* to oxygen) is very high[2], such that responses of the oxygen-sensitive glomus cells of the CB would be predicted to be insensitive to physiological levels of hypoxia within the CB (estimated to be ~60 mm Hg). However, measurements of the oxygen-dependence of changes in cytochrome a_3 absorbance, mitochondrial metabolites (NADH/NAD), mitochondrial membrane potential and calcium signals have all indicated a greater level of oxygen sensitivity in glomus cells than predicted[3-5]. Although measurements of higher than expected levels of oxygen sensitivity might simply reflect more complicated mitochondrial oxygen kinetics than those implied by MCIV properties, these measurements clearly differed between glomus cells and oxygen-insensitive but otherwise similar cells of neuronal origin[4,5]. Moreover, attempts to measure the oxygen-sensitivity of MCIV activity directly in cells also revealed greater sensitivity to hypoxia than expected from the above studies[5]. Again, this oxygen sensitivity was observed in glomus cells, but not neurons from the superior cervical ganglion[5].

It was therefore of great interest when transcriptomic analyses in glomus cells highlighted the unusually high levels of expression mRNAs encoding 'atypical' MCIV subunits including *Cox4i2*, *Cox8b* and *Ndufa4l2*, in the CB relative to neural and most other tissues[6]. In addition to the atypical mitochondrial subunits, these transcriptomic analyses demonstrated high levels of expression of *Epas1* (the gene encoding HIF-2α), which was again specific to the CB[6]. In keeping with this, HIF-2α plays a key role in CB development[7] and respiratory control[8], particularly the increased sensitivity of the CB that is observed after acclimatization to a hypoxic atmosphere.

The work by Moreno-Dominguez *et al.*[1] potentially ties together these findings on the expression of atypical mitochondrial subunits and *Epas1* in glomus cells. They demonstrated that genetic inactivation of either *Cox4i2* or *Epas1* in the CB largely ablated both the electrophysiological and ventilatory responses to hypoxia in the unacclimatized state. In mice studied at an interval of ~2 months after genetic inactivation of *Epas1*, they observed substantially reduced expression of *Cox4i2*, *Ndufa4l2* and *Cox8b* and proposed that one action of HIF-2α in the CB is to induce the expression of genes required for this atypical pattern of mitochondrial subunit expression, which underlies the special sensitivity of the organ to hypoxia. They further showed that increases in mitochondrial NADH and in reactive oxygen species (ROS) within the intermembrane space, which normally occur in hypoxia, were abolished in *Cox4i2* knock-out mice and proposed that this increase in ROS arising from a 'back-up' of electrons proximal to MCIV signals hypoxia to the effector in in glomus cells, an oxygen-sensitive K^+ channel. Though details of this process remain to be understood, the work is important in providing a molecular focus for cell-type specific mitochondrial functions that are associated with physiological oxygen-sensing responses.

Human MCIV consists of 13 subunits, 3 mitochondrially-encoded and 10 nuclear-encoded, of which COX4 is the largest. The two isoforms COX4I1 and COX4I2 arose by gene duplication at the base of vertebrate evolution. *COX4I2* expression is highly tissue-specific and its expression pattern shows an interesting correlation with organs involved in oxygen delivery or that are oxygen sensitive, including the lungs, CB, brain and fish gills[6,9]; in some cells, *COX4I2* is also induced by hypoxia. Intriguingly, another acutely oxygen-sensitive response, pulmonary vasoconstriction, fails to occur after genetic inactivation

of *Cox4i2*, although a different mode of signal transduction to the effector K^+ channels has been proposed[10].

The work provides a molecular focus for cell-type specific mitochondrial functions that are associated with physiological oxygen-sensing responses and opens many questions in CB physiology. Is enhanced COX4I2 expression (either alone or in combination with other atypical subunits) sufficient to confer oxygen sensitivity? What is the quantitative relationship between the abundance of COX4I2 and the extent of oxygen sensitivity? Is *COX4I2* a direct HIF-2 transcriptional target within the CB? Might the role of HIF-2α in increased ventilatory sensitivity to hypoxia or acclimatization reflect transcriptional induction of such targets? More broadly, how general is the association between expression of these mitochondrial proteins and physiological responses to hypoxia? How precisely is extreme oxygen sensitivity generated? Will it now be possible to generate a cellular model system in which the transduction mechanisms between the mitochondrial signal and activity of the effector channel can be interrogated? Quite possibly, after more than 80 years of investigation, we are on the threshold of gaining a precise molecular biochemical understanding of these rapid responses to hypoxia.

Fig. 1. Mitochondrial model of acute oxygen sensing in carotid body glomus cells. Schematic representation of atypical mitochondrial subunit expression (including *Cox4i2*) in mitochondria from glomus cells of the carotid body and acute oxygen sensing. Genetic inactivation of *Cox4i2* or *Epas1* in carotid body glomus cells abolishes this response.

References and Notes

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