

Carotid body oxygen sensing and adaptation to hypoxia

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Received: 4 August 2015 / Revised: 1 September 2015 / Accepted: 4 September 2015 / Published online: 16 September 2015
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Abstract The carotid body (CB) is the principal arterial chemoreceptor that mediates the hyperventilatory response to hypoxia. Our understanding of CB function and its role in disease mechanisms has progressed considerably in the last decades, particularly in recent years. The sensory elements of the CB are the neuron-like glomus cells, which contain numerous transmitters and form synapses with afferent sensory fibers. The activation of glomus cells under hypoxia mainly depends on the modulation of O₂-sensitive K⁺ channels which leads to cell depolarization and the opening of Ca²⁺ channels. This model of sensory transduction operates in all mammalian species studied thus far, including man. However, the molecular mechanisms underlying the modulation of ion channel function by changes in the O₂ level are as yet unknown. The CB plays a fundamental role in acclimatization to sustained hypoxia. Mice with CB atrophy or patients who have undergone CB resection due to surgical treatments show a marked intolerance to even mild hypoxia. CB growth under hypoxia is supported by the existence of a resident population of neural crest-derived stem cells of glia-like phenotype. These stem cells are not highly affected by exposure to low O₂ tension; however, there are abundant synapse-like contacts between the glomus cells and stem cells (chemoproliferative synapses),

which may be needed to trigger progenitor cell proliferation and differentiation under hypoxia. CB hypo- or hyper-activation may also contribute to the pathogenesis of several prevalent human diseases.

Keywords Oxygen sensing · Carotid body · Acclimatization to hypoxia · Neural crest-derived stem cells · Carotid body pathophysiology

Introduction

Oxygen (O₂) is essential for cells to generate ATP through oxidative phosphorylation. Adaptive responses to the lack of O₂ (hypoxia) have therefore evolved, particularly in mammals, to ensure O₂ supply to the most O₂-dependent organs such as the brain or the heart. Responses to hypoxia can be acute or chronic [41]. In mammalian cells, sustained hypoxia (hours or days) causes inhibition of O₂-sensitive prolyl hydroxylases, which results in the stabilization of hypoxia-inducible transcription factors, in particular HIF1 α and HIF2 α . Increased levels of HIFs modulate the expression of a broad array of genes that lead to an increased number of O₂-transporting red blood cells, non-aerobic metabolism, and the formation of new blood vessels [76]. However, survival in hypoxia requires the activation of fast, cardiorespiratory, and circulatory systemic reflexes (e.g., hyperventilation or sympathetic activation), with a time course of seconds, to increase the uptake of O₂ and its distribution to the tissues. These acute reflexes are mediated by specialized O₂-sensitive cells in the arterial chemoreceptors and other organs, which constitute the homeostatic oxygen sensing system [86]. The main arterial chemoreceptor is the carotid body (CB), a small, paired organ, located at the bifurcation of the carotid arteries, which is necessary for the adaptation of mammals (including man) to

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environments with low O₂ tension (PO₂) [86]. In addition to its critical minute-to-minute regulatory actions on the respiratory and cardiovascular systems, the CB has a fundamental role in acclimatization to hypoxia, growing in size in high altitude residents or in patients with cardiorespiratory diseases, and thereby increasing the activation of sensory fibers impinging upon the central respiratory neurons to evoke hyperventilation [1, 46]. CB hypertrophy under hypoxia occurs due to the existence within the organ of a resident population of neural crest-derived stem cells, which remain active in adult life [60]. CB changes during adaptation to hypoxia have become an area of increasing medical interest as a result of their potential involvement in tumorigenesis or in the pathophysiology of highly prevalent diseases such as obstructive sleep apnea (OSA) syndrome. In this paper, we present a summary of the cellular physiology of CB chemoreceptor cells, followed by a description of the role of CB in adaptation to chronic hypoxia and the properties of CB adult stem cells. Finally, we discuss the participation of the CB in mechanisms of disease.

Cellular physiology of O₂-sensitive carotid body glomus cells

The CB is composed of clusters of cells, called glomeruli, in close contact with a profuse network of capillaries (Fig. 1a). Each glomerulus contains several neuron-like glomus, or type I, cells which possess numerous secretory vesicles filled with neurotransmitters, in particular dopamine, ATP, acetylcholine, and several neuropeptides. These cells normally express catecholaminergic markers, such as tyrosine hydroxylase (TH). Glomus cells are O₂-sensitive presynaptic-like elements that form synaptic contacts (“chemosensory synapses”) with afferent excitatory nerve fibers of the nodose and petrosal ganglia, which terminate at the brain stem respiratory centers. Glomus cells are enveloped by the processes of glia-like, sustentacular or type II, cells which can be identified by immunostaining against glial fibrillary acidic protein (GFAP) [41, 60].

Although the chemosensory nature of the CB body has been known for almost a century, the physiology of glomus cells only begun to be explored in detail after the development of *in vitro* preparations such as enzymatically dispersed cells [19, 21, 39] or CB thin slices [56, 59], which facilitated experiments using patch-clamp techniques as well as the recording of single cell parameters by microfluorimetry, amperometry, and other methodologies. Glomus cells are excitable, contain voltage-gated Na⁺, Ca²⁺, and K⁺ channels, and can therefore generate action potentials. They have both voltage-dependent [39, 42, 63, 80] and background [7, 14] membrane K⁺ channels, which are inhibited during exposure to hypoxia. Cationic channels activated by hypoxia have also been described in glomus cells, although their role in sensory transduction needs to be confirmed [34]. Modulation of O₂-sensitive ion channels by hypoxia leads to membrane depolarization, Ca²⁺ influx through

voltage-gated Ca²⁺ channels, and transmitter release (Fig. 1b, c) [8, 84]. Among the rich variety of neurotransmitters stored in glomus cell synaptic vesicles, ATP and acetylcholine are the best candidates for the activation of the afferent sensory fibers at the chemosensory synapse [22, 85, 90]. Dopamine, which is highly abundant in glomus cells, is believed to have an inhibitory autocrine/paracrine role [4] and some neuropeptides, such as endothelin-1 (ET1), appear to be involved in the regulation of CB plasticity during exposure to sustained hypoxia [10, 59]. Interestingly, ET-1 has also been involved in the chemosensory plasticity induced by chronic intermittent hypoxia (62, 65, 71). The “membrane model” of chemotransduction, based on depolarization by hypoxia and transmitter secretion dependent on extracellular Ca²⁺ influx, is now broadly accepted and also explains the effect of other chemostimulants, such as acidosis or hypercapnia, which induce cell depolarization. CB glomus cells have also been shown to be sensitive to hypoglycemia by a mechanism independent of O₂-sensing, which involves activation of a background cationic inward current [24]. However, whether CB cells actually function as physiologically relevant glucose sensors that mediate counter-regulatory responses to hypoglycemia is still under debate [23].

Recently, human CB glomus cells have been shown to have similar electrophysiological and chemosensory properties to those in lower mammalian species [31, 54]. Interestingly, the number of TH⁺ cells in the human CB is much smaller than that in lower mammals, although this seems to be due to down-regulation of TH expression rather than an actual decrease in the number of glomus cells. Staining with antibodies against dopamine decarboxylase (DDC), another enzyme characteristic of glomus cells, demonstrates the abundance of glomeruli with DDC⁺ glomus cells in human CB tissue (Fig. 1d, e). The responsiveness of human CB cells to hypoxia, even at advanced age, is similar to that reported in other species (Fig. 1f, g) [54]. These technical advances should facilitate investigations on the potential alterations of CB function in man and their contribution to human diseases.

Despite the advances in our understanding of CB cell physiology summarized above, the mechanism whereby glomus cells actually detect changes in O₂ tension to signal membrane K⁺ channels is still unknown. This is probably a consequence of the small size of the CB, which prevents studies based on large-scale biochemical and molecular biology techniques. In addition, sensitivity to hypoxia is a labile process that is frequently altered by the vigorous enzymatic and mechanical treatment necessary to obtain dispersed glomus cells. The CB slice preparation can overcome some of these limitations, thereby facilitating the ability to conduct highly reproducible studies in animals and man [54, 59]. Although several appealing putative O₂-sensing mechanisms have been postulated to function in the CB and other peripheral chemoreceptors [20, 38, 70], none of the existing proposals has gathered solid experimental support. In some cases, it has been reported that

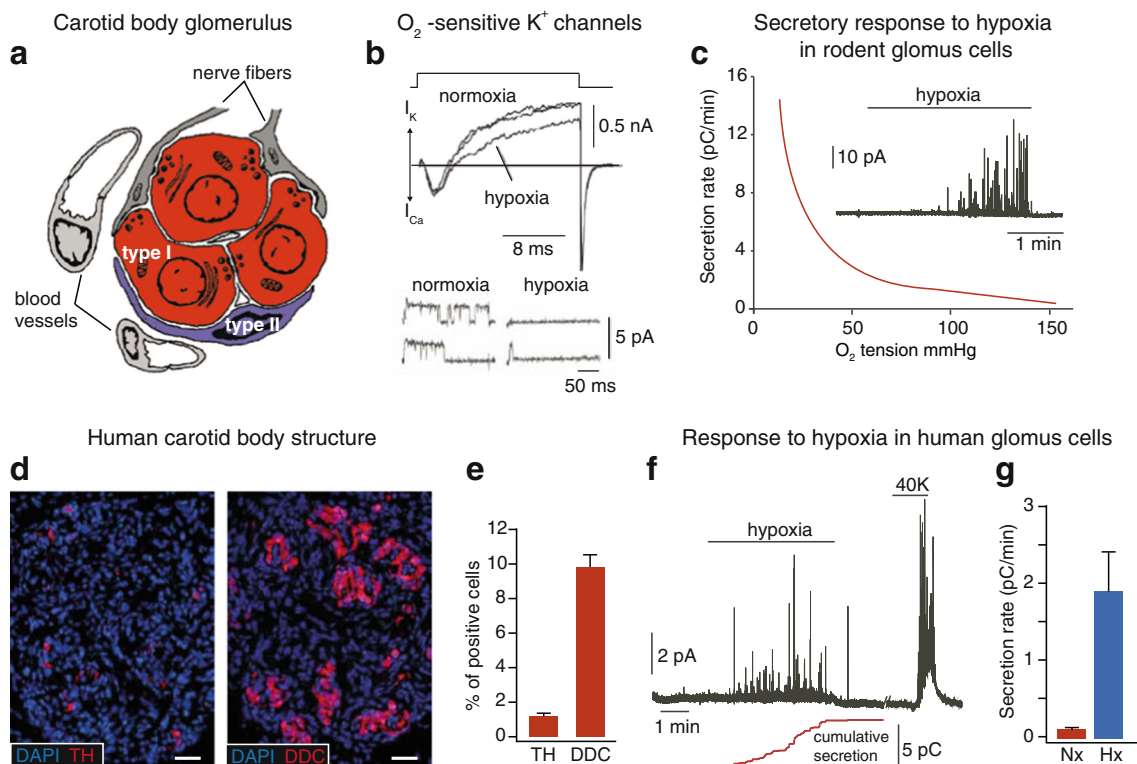


Fig. 1 Structure and function of carotid body (CB) cells. **a** Schematic representation of a CB glomerulus with indication of glomus (type I, red) and sustentacular (type II, purple) cells, blood vessels and afferent sensory nerve fibers. **b** *Top*. Ionic currents recorded from a patch-clamped rabbit glomus cell during a depolarizing pulse from -80 to $+20$ mV. Inward calcium (I_{Ca}) and outward potassium (I_K) currents are indicated. Voltage-gated sodium channels were blocked with tetrodotoxin. Note the selective reversible inhibition of the K^+ current by exposure to hypoxia (switching from an O_2 tension of 150 to 15 mmHg). **b** *Bottom*. Inhibition

of single K^+ channel activity recorded in an excised membrane patch exposed to hypoxia. **c** Catecholamine secretion from rodent glomus cells as a function of O_2 tension. The inset illustrates the secretory response of a cell to hypoxia as monitored by amperometry in CB slices. Modified from [36]. **d, e** Number of tyrosine hydroxylase (TH) and dopamine decarboxylase (DDC) positive cells in human CB slices. Scale bar 50 μ m. Quantification in **e** was obtained from 23 (DDC) and 28 (TH) donors from 10 to 67 years. **f, g** Secretory activity of cells in human CB slices in response to hypoxia (~ 10 mmHg) and 40 mM K^+ . Modified from [48]

the responsiveness to hypoxia is unaffected in genetically modified animals lacking putative O_2 -sensor molecules (i.e., NADPH oxidase or hemoxygenase 2) [27, 55]. In other cases, pharmacological analyses have cast doubts on the need for the proposed O_2 -sensing molecules or messengers, such as NO or H_2S , in acute responsiveness to hypoxia. O_2 -sensitive prolyl hydroxylases are known to have a fundamental role in adaptation to chronic hypoxia. However, although this system might be necessary for the maintenance of CB homeostasis, it does not seem to directly participate in acute O_2 sensing [56]. Nonetheless, prolyl hydroxylation can occur in some ion channel classes that are expressed in chemoreceptor cells [81]. Therefore, it is possible that the function, expression and/or membrane distribution of these channels are modulated by O_2 -dependent hydroxylation, thereby allowing them to participate in the adaptation to hypoxia.

A classical view is that mitochondria are involved in acute O_2 sensing due to the exquisite sensitivity of CB cells to cyanide and other electron transport chain (ETC) inhibitors [9, 18, 53, 88]. The term “metabolic hypothesis”, coined to stress the

role played by mitochondria in O_2 -sensing [49, 51], is not in contradiction with the membrane model of chemotransduction discussed above as mitochondria utilize O_2 and therefore could detect changes in O_2 availability and signal the membrane channels in response to hypoxia. Indeed, modulation by hypoxia of mitochondrial reactive oxygen species (ROS) production has been proposed to be an essential component of O_2 sensing by cells, particularly in pulmonary arterial myocytes [26, 48, 86]. However, the potential mechanisms whereby these organelles sense O_2 and signal membrane K^+ channels are unknown. As mitochondrial parameters (e.g., mitochondrial membrane potential or NAD(P)H autofluorescence) in chemoreceptor cells are highly sensitive to changes in PO_2 , it was proposed that Ca^{2+} release from these organelles is the signal that triggers secretion during exposure to hypoxia [18]. Although this model lost support once it was established that the secretory response of chemoreceptor cells to a decrease in PO_2 depends on extracellular Ca^{2+} influx [8, 84], it still remains possible that hypoxic mitochondria could provide a modulatory signal that affects ion channels and thus alters the membrane

potential of glomus cells. In accord with this concept, activation of CB cells by ETC inhibitors depends on extracellular Ca^{2+} influx [53, 88]. A new perspective on the role of mitochondria as O_2 sensors resulted from experiments showing that rotenone, an inhibitor of mitochondrial complex I (MCI), selectively occludes responsiveness to hypoxia in CB glomus [24, 53] and adrenomedullary (AM) chromaffin [82] cells without altering the response to hypoglycemia. These pharmacological data led us to postulate that a rotenone-binding molecule is involved in CB O_2 sensing [53], although definitive experimental proof is still to be obtained.

Carotid body and acclimatization to hypoxia

The CB is thought to play an essential role in acclimatization to chronic hypoxia, a frequent condition in the human population that affects millions of people who live at or travel to high altitudes and are therefore exposed to low atmospheric air pressure and decreased O_2 diffusion into the blood. Hypoxemia is also highly prevalent in medical disorders, such as chronic obstructive pulmonary disease (COPD), that cause a reduction of the O_2 exchange capacity between the alveolar gas and the pulmonary capillaries [30, 79]. Adaptation to hypoxic environments depends on the hypoxic ventilatory response (HVR) [30, 69], a respiratory reflex that disappears in patients who have undergone surgical CB resections (most commonly due to tumors or asthma treatment) [83]. These individuals appear to live unaffected in normoxic environments, although disturbances during sleep and cases of sudden death have been attributed to a lack of functional chemoreceptors. In addition, it is known that the CB grows in individuals who are exposed to sustained hypoxemia for several days. This adaptive response increases the excitatory signals reaching the brain stem respiratory center to maintain hyperventilation [1, 30, 46].

Intolerance to hypoxia after disruption of the carotid body-adrenal medulla axis

Investigations of the role played by the CB in adaptation to hypoxia have proven difficult due to a lack of appropriate experimental models. The CB is generated during embryogenesis by the migration of sympathetic precursor cells from the superior cervical ganglion to the primordial carotid artery [28], and mutations of genes that prevent either carotid artery formation or sympathetic development are known to result in CB defects. However, most of the animals affected by these mutations die during embryonic life or shortly after birth and therefore cannot be studied in adulthood [13, 33]. Recently, we generated a new animal model (denoted as TH-VHL^{KO} mice) in which ablation of the gene encoding the von Hippel-Lindau (Vhl) protein is restricted to TH⁺ catecholaminergic cells [43]. Vhl has a well-established role as a

substrate recognition subunit of an ubiquitin ligase complex that targets HIF α for proteasomal degradation [44]. However, other functions of Vhl are not fully understood, and mutations of the Vhl gene are associated with the appearance of tumors. In normoxic conditions, our Vhl-deficient animals show full development of the brain and other organs and normal physiological functions although they exhibit hypotrophy of the CB, AM, and peripheral sympathetic system. TH-VHL^{KO} mice do not have an HVR (Fig. 2a) and exhibit a striking intolerance to sustained hypoxia (Fig. 2b). Exposure of the animals to even mild hypoxia (14 % O_2) causes strong erythropoietin induction and erythropoiesis, which within a few days is followed by splenomegaly, severe pulmonary hypertension, and right cardiac hypertrophy leading to death (Fig. 2c–e) [43]. These observations demonstrate the absolute necessity of peripheral chemoreceptor cells in the CB body and the AM for acclimatization and survival in hypoxic conditions. They also indicate that the TH-VHL^{KO} mice provide an optimal model in which to study the consequences of functional inhibition of peripheral chemoreceptors throughout life. In this way, investigations of the early signs of hypoxia intolerance or the identification of biomarkers sensitive to maladaptation to hypoxia can be conducted. This could help to prevent hypoxia-associated morbidities affecting the brain or cardiorespiratory system, which are highly prevalent in susceptible individuals [74, 79, 87].

Carotid body stem cell niche

An intriguing property of the CB is its ability to grow in response to chronic hypoxia (Fig. 3a). Although this response was first described many years ago [1, 30, 46, 69], the underlying mechanisms have remained largely unstudied [10]. It has recently been shown that CB plasticity during sustained hypoxia depends on the existence in this organ of a population of multipotent adult neural crest-derived stem cells, which are the sustentacular or type II cells [60]. These cells are quiescent in normoxia but during exposure to hypoxia, lose their characteristic GFAP⁺ phenotype in parallel with the appearance of a progressive increase in the number of BrdU⁺ proliferating cells within the CB. Cell fate mapping experiments *in vivo* have shown that type II cells change their phenotype in response to hypoxia and are converted into proliferating (nestin⁺) progenitors, which in turn differentiate into new TH⁺ glomus cells [60]. Nestin⁺ progenitors can also give rise to smooth muscle and endothelial cells [43, 60]. These changes in the CB stem cell niche can also be observed *in vitro* using neurosphere assays. CB neurospheres are cell colonies that normally derive from a single stem cell and are composed of a core of nestin⁺ proliferating progenitors surrounded by differentiating TH⁺ cells budding out of the core. The newly generated TH⁺ cells form blebs attached to the neurosphere core, which grow in size with time in culture (Fig. 3b). Mature

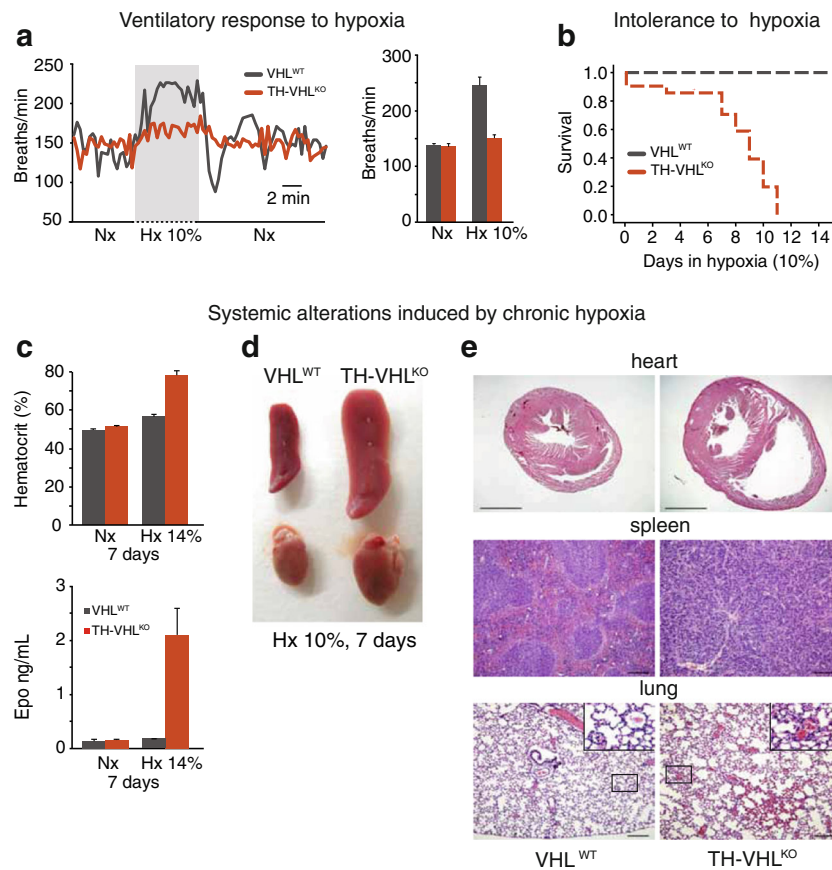


Fig. 2 Responses to hypoxia in mice with atrophy of the carotid body/adrenal medulla axis. **a** Plethysmograph recordings showing changes in respiration rate during exposure to normoxia (Nx, 21 % O₂) and hypoxic (Hx, 10 % O₂) environments in control (VHL^{WT}) and Vhl-deficient (TH-VHL^{KO}) mice. **b** Kaplan–Meier survival curves comparing VHL^{WT} and TH-VHL^{KO} mice after exposure to chronic hypoxia (10 % O₂). **c** Hematocrit and plasma erythropoietin (Epo) levels in animals maintained in chronic hypoxia (14 % O₂). **d** Enlargement of the spleen (*top*) and heart

(*bottom*) of TH-VHL^{KO} mice after exposure to chronic hypoxia (10 % O₂). **e** Hematoxylin/eosin staining of thin sections of the heart, lung, and spleen of VHL^{WT} and TH-VHL^{KO} mice maintained for 7 days in chronic hypoxia. Note the marked increase in size of the right ventricle, as well as pulmonary edema and changes in the histological architecture of the spleen. Scale bars: heart, 2 mm; spleen and lung, 0.2 mm. Modified from [39]

glomus cells derived from CB stem cells have similar functional properties to normal glomus cells in situ. They have voltage-gated ion channels, can release dopamine and other transmitters in response to hypoxia or hypoglycemia, and synthesize glial cell line-derived neurotrophic factor, which is highly expressed in adult CB cells [60]. In many aspects, differentiation of neuron-like glomus cells from GFAP⁺ stem cells in the CB resembles the organization of the central neurogenic centers located in the subventricular zone (SVZ) or the hippocampus. In both cases, there are neural stem cells with a quiescent astrocytic (GFAP⁺) phenotype that are converted to nestin⁺ transient amplifying progenitors which in turn differentiate into neuroblasts giving rise to mature neurons [60]. Support for this model has also come from recent studies on TH-VHL^{KO} mice, which have a strong reduction in the number of TH⁺ cells in the carotid bifurcation (Fig. 3c, *top*) but contain a large number of GFAP⁺ progenitor cells in apparently good condition (Fig. 3c, *bottom*). Indeed, neurosphere assays have confirmed the increased number of

CB stem cells in these animals (Fig. 3d, e) and the loss of TH⁺-differentiated glomus cells (Fig. 3d, e). Newly generated TH⁺ cells in Vhl-deficient neurospheres appear to die as soon as TH-dependent Cre recombinase is induced and the *Vhl* gene is ablated (Fig. 3f) [43]. Multipotent neural stem cells have also been found in other parts of the peripheral nervous system, such as the enteric nervous system or the dorsal root ganglion. However, whether these cells participate in the maintenance of homeostasis of the organs in which they reside has not been established [29]. In addition to the growth of the CB parenchyma, sustained hypoxia also leads to an increase in CB glomus cell excitability due to, among other factors, an increase in Ca²⁺ current amplitude and a decrease in K⁺ channel expression [10, 68].

*O*₂-sensitive chemoproliferative synapses

In contrast with the exquisite sensitivity of the CB to hypoxia, CB stem cell proliferation in vitro is virtually unaffected by

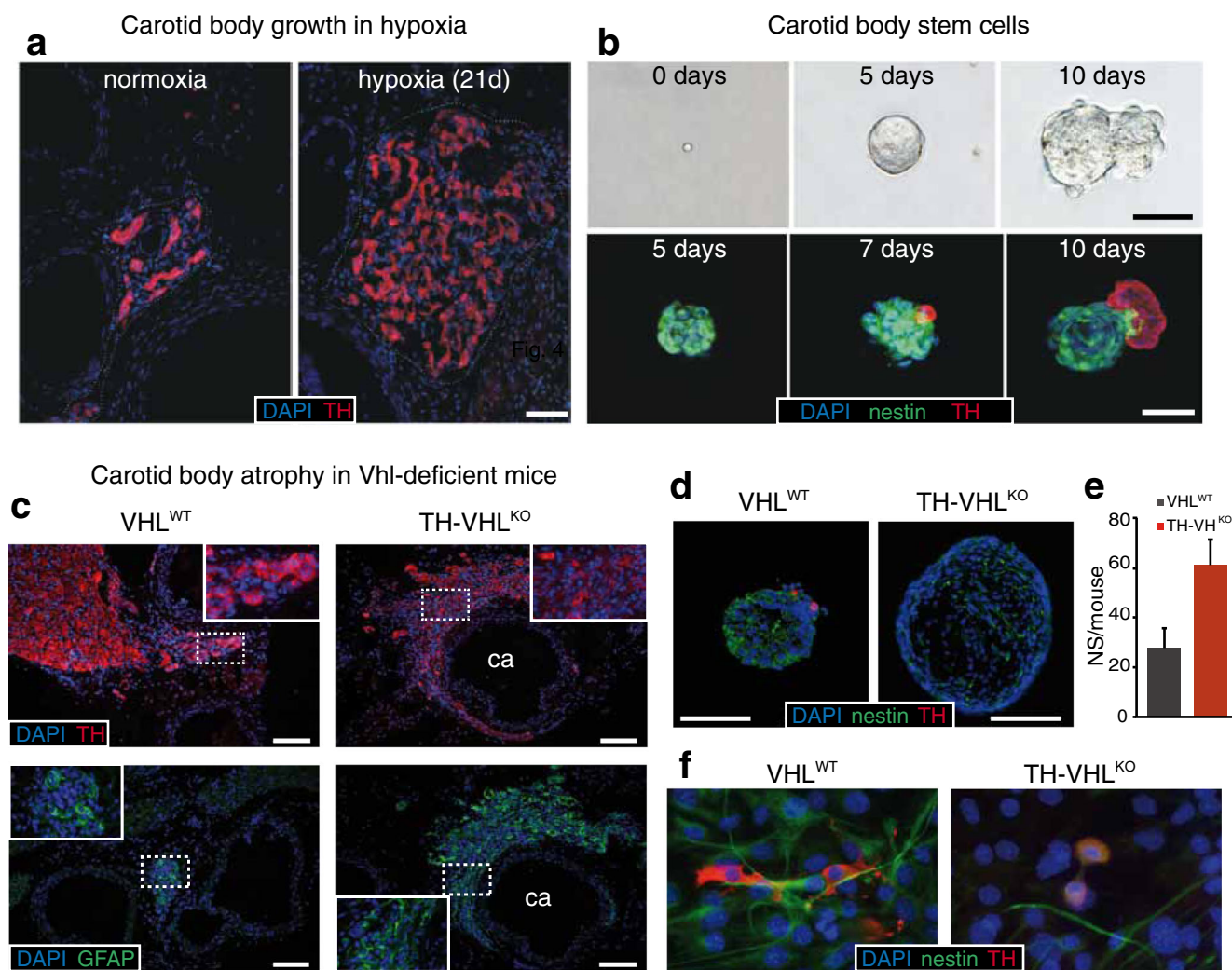


Fig. 3 Carotid body (CB) growth in chronic hypoxia and CB stem cells. **a** Increase in size of CB parenchyma in mice exposed to 10 % O₂ for 21 days. **b** Sequential photographs of a colony illustrating the formation of a typical CB neurosphere from a single CB stem cell (*top*) and time course of rat CB neurosphere formation *in vitro* (*bottom*). Appearance of blebs of (TH+) glomus cells is preceded by the formation of the neurosphere core containing nestin+progenitors. Scale bars, 50 μ m. Modified from [54]. **c** *Top*. Marked decrease in the number of TH+ cells (superior cervical ganglion and carotid body) in the carotid bifurcation in

TH-VHL^{KO} mouse. **c** *Bottom*. Increase in the number of GFAP+ (green) type II cells in the carotid bifurcation in TH-VHL^{KO} mice. The areas inside the rectangles are shown at a higher magnification in the insets. *ca* carotid artery. **d**, **e** Comparison of neurosphere composition and size (**e**), and number of neurospheres (**f**) in CB from control (VHL^{WT}) and TH-VHL^{KO} mice. **f** Differentiation of neuron-like glomus cells in neurospheres from control and TH-VHL^{KO} mice. Note the appearance of pyknotic TH+ cells in TH-VHL^{KO} preparations. Scales bars 100 μ m (**c**, **e**), 2 μ m (**d**), 10 μ m (**f**). Modified from [39]

lowering O₂ tension. However, hypoxia strongly induces TH expression and promotes differentiation to glomus cells (Fig. 4a–d). These data are in accord with recent reports suggesting that hypoxia is a critical condition of the adult and embryonic stem cell niches. Quiescent somatic stem cells seem to maintain a predominantly anaerobic metabolism, which helps to preserve them from excessive production of ROS and other stressors [50, 78]. Resistance to hypoxia is a general property of neural stem cells as it has been found that the survival and proliferation of stem cells in the SVZ is not affected by a reduction in O₂ tension from 21 to 1 % [12]. In line with these *in vitro* observations, CB growth is not induced

by systemic administration of dimethylxalylglycine (DMOG), an inhibitor of prolyl hydroxylases that mimics hypoxia and stabilizes HIFs in all tissues [5, 67]. Whereas mice treated with DMOG exhibit induction of classical HIF-dependent genes, such as erythropoietin and vascular endothelial growth factor, DMOG fails to induce CB cell proliferation (Fig. 4e–g). Taken together, these findings suggest that hypoxia does not directly activate the proliferation of CB stem cells. Indeed, morphological and functional data have indicated the existence of synaptic contacts between the O₂-sensitive glomus cells and the GFAP+ stem cells, thus providing the structural basis for O₂-sensitive chemoproliferative synapses

Proliferation of carotid body progenitor cells is unaffected by hypoxia

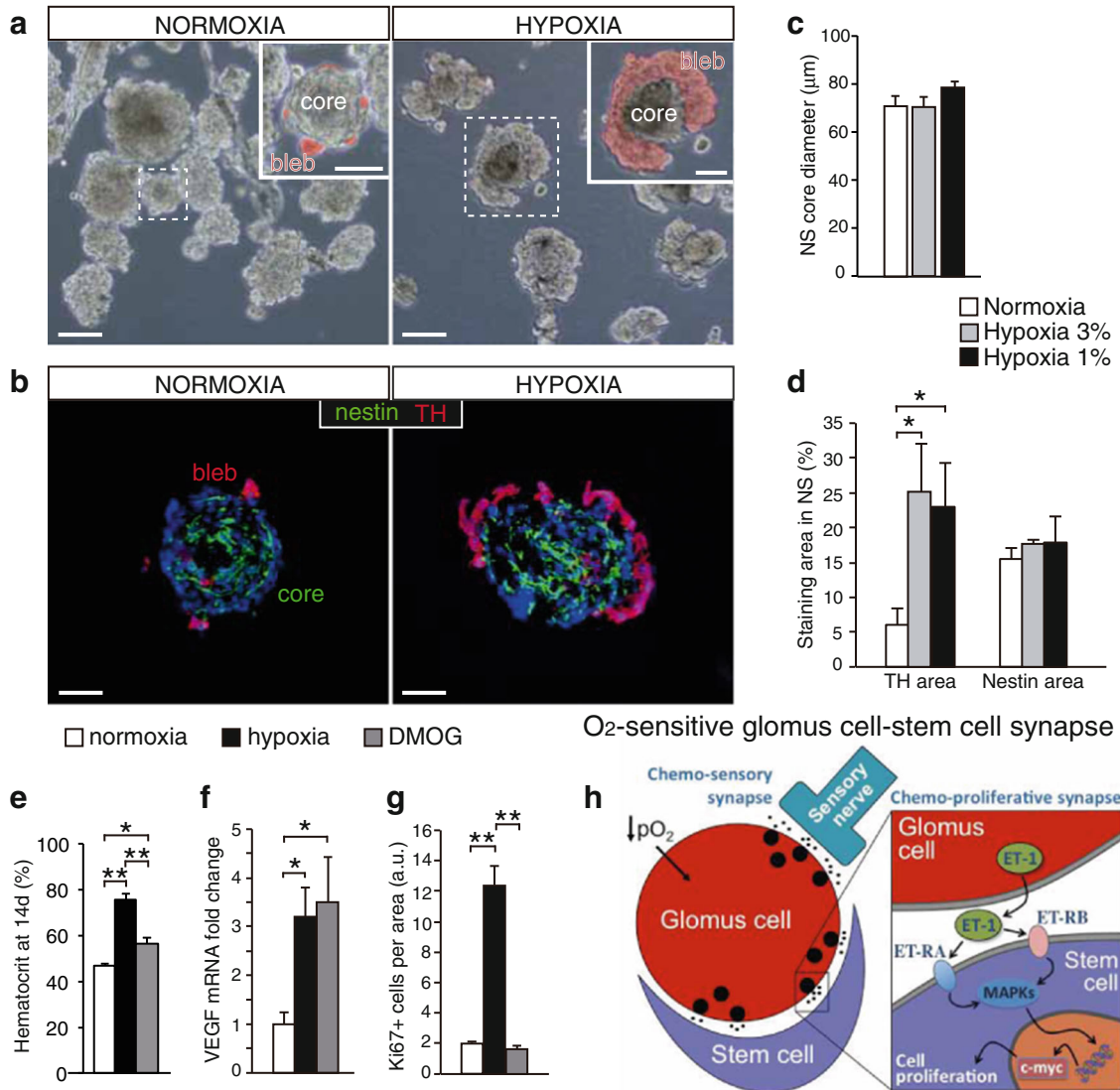


Fig. 4 Resistance of carotid body stem cells to hypoxia and O₂-sensitive glomus cell-stem cell synapse. **a** CB neurospheres (NS) cultured under normoxia (21 % O₂) or hypoxia (3 % O₂). Differentiating blebs are colored in red in the insets. **b** Representative NS with a core of nestin+ progenitors and blebs of TH+ cells. **c** NS core diameter in cultures grown at different levels of hypoxia. **d** Measurement of nestin+ or TH+ staining area in normoxic and hypoxic NS sections. Note that the TH+ area clearly

increased under hypoxia, whereas the nestin+ area did not change. **e–g** Quantification of hematocrit (**e**), brain mRNA levels of vascular endothelial growth factor (VEGF) (**f**) and proliferating (Ki67+) cells in the carotid body (**g**) in rats maintained in normoxia (21 % O₂), hypoxia (10 % O₂) or in normoxia under DMOG treatment. Scale bars **a** 100 μm (50 μm in insets) **b** 50 μm. Modified from [59]

which trigger CB growth in sustained hypoxia (Fig. 4h). Among the putative chemical transmitters in the glomus cell-stem cell synapse, we have identified ET1, a vasoactive and pro-proliferative agent involved in neural crest progenitor specification and migration [6, 77] that is synthesized in glomus cells [10, 47, 58, 67, 68]. ET1 receptors, in particular subtype B, are also highly expressed in type II cells [67].

The picture that emerges from these studies is that the CB glomerulus is a highly sophisticated organoid-like structure with complex autocrine and paracrine interactions among its different cellular components. The O₂-sensitive glomus cells

are the central elements that, in response to hypoxia, mediate the activation of afferent sensory fibers (chemosensory synapse), thereby evoking an acute hyperventilatory response. In parallel, glomus cells trigger the proliferation of quiescent stem (type II) cells (“chemoproliferative synapses”) that initiates CB growth (see Fig. 4h). In addition, to these synapses, recent data have suggested even more elaborate interactions among cells within the CB glomerulus. Glomus cells are known to release ATP in response to hypoxia, which activates metabotropic receptors in type II cells [89]. In turn, activated type II cells appear to release ATP through large pannexin

channels, increasing the excitatory response. All these responses seem to be further modulated by the interaction between the different chemical signals present in the CB and the afferent autonomic innervation [52].

Carotid body and human disease

Although the main biomedical interest in CB physiology derives from its role in the control of respiration, there is mounting evidence to indicate that the CB could be involved in the pathogenesis of several human diseases, some of which are highly prevalent. CB sensitivity to hypoxia develops during the early postnatal period and correlates with enhanced chemosensitivity in glomus cells. This process is important in the newborn given that, in addition to increasing ventilation and sympathetic tone, activation of the CB facilitates arousal from sleep and the switch from nasal to oral breathing. Defects in CB development or maturation have been associated with several neonatal respiratory deficiencies, such as sudden infant death or congenital central hypoventilation syndrome [40, 86]. CB denervation around the time of birth produces respiratory disturbances, exposing the newborn to respiratory instability and unexpected death. However, in several adult animal species, the hyperventilatory response to hypoxia, which is abolished by CB removal, is totally or partially re-established several months after the surgery (possibly due to the activation of other chemoreceptors). In man, CB resection due to tumor surgery or treatment for asthma results in complete and sustained lack of hypoxia responsiveness, particularly during sleep [83]. In recent years, alterations of CB function in adult life have been gaining increasing interest due to their participation in the pathogenesis of human pathologies, such as neural crest-derived tumors and the development of chronic neurogenic hypertension.

Carotid body tumorigenesis

Although relatively uncommon, CB tumors (chemodectomas or paragangliomas) are normally associated with medical syndromes presenting tumorigenesis in organs of the peripheral nervous system. These are mostly benign tumors that, besides the symptoms due to local compression, can also produce systemic hypertension [73]. Chemodectomas present with CB hypertrophy and cellular hyperplasia/anaplasia mainly affecting TH+ glomus cells although type-II cells are also affected. The grade of malignancy of CB paragangliomas appears to be inversely associated with the number of GFAP+ type-II cells, a fact that could indicate that deregulation of CB progenitors (with a type-II cell phenotype) participates in tumorigenesis. The most frequent cause of chemodectoma is hereditary CB paraganglioma due to loss of function mutations in *SDHD*, a gene that encodes the small membrane anchoring subunit of succinate dehydrogenase in mitochondrial complex II [3]. In affected individuals

(heterozygous for mutated *SDHD*), the accidental loss of the normal allele (loss of heterozygosity, LOH) triggers tumor initiation. Indeed, tumorigenesis is favored in conditions that stimulate CB cell proliferation, such as living at high altitudes [2]. The pathogenesis of hereditary paragangliomas is unknown, although it has been proposed that the accumulation of succinate in the LOH-affected cells inhibits prolyl hydroxylases and stabilizes HIFs, thus causing a “pseudohypoxic status” leading to abnormal cell proliferation. Other molecular processes, such as oxidative stress or chromatin remodeling have also been proposed to play a causative role. Although bi-allelic ablation of *SdhD* causes embryonic lethality, heterozygous *SdhD* knockout mice are apparently normal and simply show a mild CB hypertrophy with normal sensitivity to hypoxia and no histological signs of tumorization [66]. Moreover, conditional knockout animals with *SdhD* ablation restricted to catecholaminergic (TH+) cells do not develop tumors but rather display a marked cell loss in the CB and other peripheral sympathetic organs [17]. It seems, therefore, that although *SDHD* is considered a “tumor suppressor gene” in humans, its role in tumor generation is not as direct as previously thought. It is possible that in addition to the *SDHD* mutation, additional “hits” are necessary to trigger tumor initiation. The possibility that these unknown hits are favored by chronic hypoxia (lasting months or years) is currently under study in heterozygous *SdhD* knockout mice.

Tumors of neural crest lineage, such as CB paragangliomas and AM pheochromocytomas, are also frequent in some forms of VHL disease, a hereditary syndrome caused by mutations in the *VHL* gene and characterized by the occurrence of tumors in multiple tissues [25, 32]. It has been suggested that VHL might participate in the molecular cascade leading to physiological apoptosis of sympathetic progenitor cells and that impairment of this protein could predispose to tumorigenesis in adulthood [36]. In contrast with this proposal, we have found that *Vhl* deletion in mice results in sympathoadrenal cell loss and impairment of survival of stem cell-derived catecholaminergic glomus cells [43] (see Fig. 3). The data indicate that *Vhl* is not only necessary for the survival of sympathoadrenal cells during development but is also required for the maintenance (full differentiation and survival) of these cells in adulthood. Interestingly, bi-allelic loss of VHL does not seem to produce pheochromocytomas or paragangliomas, and most of these tumors appear in patients with missense mutations of the VHL gene [36]. Hence, it is possible that pheochromocytomas/paragangliomas are generated by gain-of-function VHL mutations that, in the AM/CB setting, lead to abnormal cell proliferation [43]. It is therefore clear that *Vhl* does not act as a classical tumor suppressor gene in CB cells.

Carotid body over-activation

In addition to its critical role in adaptation to acute and chronic hypoxia, substantial evidence suggests that the CB can also

have maladaptive effects. An exaggerated CB chemoreflex is known to contribute to the autonomic imbalance (tonic sympathetic over-activation) characteristic of several highly prevalent disorders, such as OSA, chronic heart failure (CHF), hypertension, obesity, metabolic syndrome, and diabetes [16, 61, 72, 75]. Recently, ablation of the CB was proposed for the treatment of refractory neurogenic hypertension [45]. The mechanisms underlying the changes in the CB-mediated chemoreflex in these pathologies seem to be intrinsic to the CB although their exact nature is as yet poorly known. In OSA, repeated episodes of intermittent hypoxia alter CB glomus cell excitability and sensitivity to lowered PO_2 [11, 57, 64]. In addition, several groups have shown that the enhancement of CB activity in animals subjected to chronic intermittent hypoxia depends on oxidative stress-mediated alteration of ET-1 and angiotensin II signaling [15, 35, 62, 65, 71]. In CHF, a progressive sustained reduction of blood flow (even in the presence of a normal arterial PO_2) seems to be the cause of CB activation, as this organ normally receives an extraordinarily high arterial blood supply. Among other factors, alterations of angiotensin II-dependent modulation of O_2 -sensitive membrane K^+ channels in glomus cells or dysfunction of redox-mediated pathways have been proposed to have an important pathogenic role in CB over-activation in CHF models [75]. Insulin has been proposed to be an intermittent hypoxia-like factor that stimulates CB activity [37, 72]. Thus, increased CB-dependent sympathetic outflow is considered to play a key role in metabolic syndrome and in the development of hypertension, diabetes, and their associated morbidities.

Conclusions and perspectives

The CB is the principal arterial chemoreceptor that mediates the fast reflex cardiorespiratory response to hypoxia. Glomus cells, the O_2 -sensing elements in the CB, contain O_2 -sensitive K^+ channels, which are inhibited by hypoxia, thereby triggering cell depolarization, Ca^{2+} influx, and activation of the chemosensory synapse connecting the CB with the brainstem respiratory center. Other O_2 -sensitive ion channel classes (e.g., non-selective cation channels) may also contribute to this response in particular during long hypoxic exposures. This membrane model of sensory transduction, generated from data obtained in lower mammalian species, has also been shown to be applicable to human CB cells. However, despite considerable research effort, the mechanisms whereby changes in O_2 tension are detected by membrane channels are still unknown. Currently, there are several appealing proposals but, as yet, none is supported by sufficient experimental evidence. As the cellular processes underlying acute O_2 -sensing are fundamental to our understanding of hypoxia and O_2 homeostasis, we expect that they will soon be elucidated. CB-

dependent hyperventilation is necessary for acclimatization to sustained hypoxia, and CB hypertrophy is an adaptive response that is characteristic of high altitude residents or patients with diseases that compromise alveolar gas exchange. Recent work has proven that animals with atrophy of the CB-AM axis survive well in normoxia but show an absolute intolerance to even mild hypoxia, presenting exaggerated erythrocytosis, pulmonary edema, and right heart dilation leading to impending death. It will be important to investigate whether CB dysfunction can explain cases of respiratory depression (e.g., during anesthesia or opioid treatment) or disacclimatization to hypoxia in man. CB growth, a classic response to chronic hypoxia, is an intriguing property that is unusual for an organ of the peripheral nervous system. The mechanisms of CB plasticity are still not known in detail, but some of them are based on the activation of a resident population of adult neural crest-derived multipotent stem cells, which have a type II (glia-like) cell phenotype. Under hypoxia, CB stem cells are converted into nestin+ progenitor cells, which in turn can give rise to glomus cells with normal chemosensitive properties as well as smooth muscle and endothelial cells. CB neural stem cells are the only ones in the adult peripheral nervous system that have a recognized physiological role. Interestingly, CB stem cells are insensitive to hypoxia, and their activation upon exposure to low PO_2 seems to depend on the stimulation of glomus cells, which form abundant synaptic-like contacts with type II cells. Among the neurotransmitters involved in these O_2 -sensitive glomus cell-stem cell chemoproliferative synapses, ET1 appears to have a prominent role. Therefore, under hypoxia O_2 -sensitive glomus cells activate the chemosensory synapse to produce an acute hyperventilatory response and the chemoproliferative synapse to trigger stem cell proliferation and differentiation. The fact that these two synapses have different neurochemical properties makes pharmacological modulation of CB expansion in chronic hypoxia possible without affecting the acute chemosensory response. The chemical synapses existing between the glomus cell and the sensory fibers or the stem cells are examples of the complex reciprocal paracrine interactions that exist among the various elements of the CB glomerulus. Unraveling the functional topology within the CB glomeruli may help us to understand organizational principles in the peripheral and central nervous systems. Advances in CB cell physiology can now provide valuable information on the pathogenesis of diseases presenting hypo- or hyper-function of the CB chemoreceptors. CB tumors, although relatively rare, provide an excellent model for investigating the molecular basis of tumorigenesis in peripheral neural tissues. CB over-activation, secondary to chronic intermittent hypoxia, chronic cardiac failure, or diabetes, is an essential component of the exaggerated sympathetic outflow that occurs in these pathologies. Selective functional CB inhibition could therefore help to improve the prognosis in these diseases and associated

comorbidities, while avoiding the need for surgical interventions.

Acknowledgements This work has been supported by grants from the Botín Foundation, the Spanish Ministries of Health and Science, and the European Research Council (European Union).

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