Archives of Biochemistry and Biophysics 534 (2013) 3–10

Contents lists available at SciVerse ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Review

Pluripotent stem cells as a model to study oxygen metabolism in neurogenesis and neurodevelopmental disorders

Bruna da Silveira Paulsen [a,1], Mariana Souza da Silveira [b,1], Antonio Galina [c], Stevens Kastrup Rehen [a,*]

[a] Laboratório Nacional de Células-Tronco Embrionárias, Instituto de Ciências Biomédicas, UFRJ, Rio de Janeiro, RJ 21941-913, Brazil
[b] Laboratório de Neurogênese, Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, RJ 21941-902, Brazil
[c] Laboratório de Bioenergética e Fisiologia Mitocondrial, Instituto de Bioquímica Médica, UFRJ, Rio de Janeiro, RJ 21941-590, Brazil

ARTICLE INFO

Article history:
Available online 27 October 2012

Keywords:
Neuronal differentiation
ROS
Oxidative stress
hiPSCs
Modeling diseases
Schizophrenia
Oxygen

ABSTRACT

Reactive oxygen species (ROS) and oxygen (O_2) have been implicated in neurogenesis and self-renewal of neural progenitor cells (NPCs). On the other hand, oxidative unbalance, either by an impairment of antioxidant defenses or by an intensified production of ROS, is increasingly related to risk factors of neurodevelopmental disorders, such as schizophrenia. In this scenario, human induced pluripotent stem cells (hiPSCs) emerged as an interesting platform for the study of cellular and molecular aspects of this mental disorder, by complementing other experimental models, with exclusive advantages such as the recapitulation of brain development. Herein we discuss the role of O_2/ROS signaling for neuronal differentiation and how its unbalance could be related to neurodevelopmental disorders, such as schizophrenia. Identifying the role of O_2/ROS in neurogenesis as well as tackling oxidative stress and its disturbances in schizophrenic patients' derived cells will provide an interesting opportunity for the study of neural stem cells differentiation and neurodevelopmental disorders.

Introduction

The importance of O_2/ROS signaling for neurogenesis

The culture of neural progenitor cells (NPCs)[2] is an essential tool for assessing mechanisms controlling differentiation in the nervous system [1]. Human NPCs can be obtained from a brain biopsy or differentiated from pluripotent stem cells. Regardless of their source, one of the main challenges in this field is to mimic *in vitro* neural development as similar as possible to the *in vivo* situation. Much has been done in order to confirm similarities between cells growing in dishes and within the brain, while *in vitro* models have evolved as reliable tools for studying cellular and molecular aspects of neural differentiation [2].

During early development, oxygen (O_2) levels, which are low in embryo, control important events, such as formation of placenta, vascular system and skeleton [3,4]. After birth, O_2 demands, consumption and flow vary amongst organs, affecting its dissolved concentration, despite the fact that they are usually much lower than values expected in the atmospheric air (~20.8% or 21 kPa) [1]. Likewise, in spite of being conserved amongst the central nervous system (CNS) of mammalian species, values of partial oxygen pressure (pO_2) are also known to vary in brain regions [5].

Actually, this variation seems to occur since the beginning of development, and may influence cell fate in a gradient manner. Consequently, O_2 has been considered as a morphogen, which promotes regulatory roles in diverse pathways controlling also neural differentiation [4]. During the early stages of neurodevelopment, as it happens with the embryo as a whole, the brain is also submitted to a hypoxic condition. This is extremely important, as low-O_2 levels regulate several essential genes that govern diverse physiological events [6]. On the other hand, in an abnormal range, hypoxia could induce an incorrect regulation of neural genes, leading to a pathological condition that is also evidenced by epidemiological data, which correlate hypoxia with neuronal dysfunction or subtle damage [6].

Although the exact mechanism involved in ROS-mediated neurogenesis remains unclear, reactive oxygen species (ROS) generation could be closely related to alterations in O_2 levels and growing evidences also indicate them as hub regulators of various

* Corresponding author.
E-mail address: srehen@lance-ufrj.org (S. Kastrup Rehen).
[1] These authors contributed equally to this work.
[2] *Abbreviations used:* ROS, reactive oxygen species; NPCs, neural progenitor cells; hiPSCs, human induced pluripotent stem cells; NOX, NADPH oxidase; HCS/HTS, high-content screening/high-throughput screening; CNS, central nervous system; NGF, nerve growth factor; NRG, neuregulin; FGF2, fibroblast growth factor 2; RA, retinoic acid; SVZ, subventricular zone; ESCs, embryonic stem cells; hESCs, human ESCs; ROS^{lo}, lower levels of ROS; ROS^{hi}, higher levels of ROS; BDNF, brain-derived neurotrophic factor; DISC1, disrupted in schizophrenia 1; AKT1, V-Akt murine thymoma viral oncogene homolog 1; CNR1, Cannabinoid receptor 1; COMT, Catechol-O-methyltransferase; GAD1, Glutamate decarboxylase; IL-10, Interleukin-10; NRG1, Neuregulin-1; CxHRNA7, Alpha-7 nicotinic ACh receptor.

0003-9861/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.abb.2012.10.009

processes and pathways during neurodevelopment [7,8]. Indeed, it was shown that endogenously produced ROS activate PI3 k/Akt, p38 MAPK and ERK signaling during neural differentiation *in vitro* [7,8]. Factors related to neuronal maturation, such as nerve growth factor (NGF), neuregulin (NRG), fibroblast growth factor 2 (FGF2) and retinoic acid (RA), are also known to increase ROS generation [9–12]. Moreover, administration of antioxidants or ROS inhibitors could abrogate differentiation, suggesting an important role of these reactive species [9,10,12,13]. However, data suggesting an influence of pO_2 and ROS in neurogenesis are not exclusive to early stages of development.

It has been demonstrated that pO_2 may also vary in postnatal neurogenic niche so as to regulate quiescence and differentiation of those cells. In the subventricular zone (SVZ), a close association between neural stem cells and vasculature is believed to facilitate the traffic of O_2, which in turn influences stem cell renewal and neurogenesis. It is proposed that quiescent stem cells are activated to self-renew when there is a reduction in O_2 tension, giving rise to transit-amplifying cells that eventually differentiate in regions where this tension is higher. This can be modulated by cellular properties that control the transfer of this gas or by the release of growth factors that integrate with O_2 response [1]. Accordingly to *in vivo* observations (reviewed by [1,14]), conditions of low-O_2 tension *in vitro* can also stimulate NPCs to proliferate and self-renew [7,15].

Since it is known that low-O_2 stimulates ROS generation [7,16], it is expected that a reduction of O_2 levels associated to self-renew stimulus will happen concomitantly to an increase of ROS. *In vivo* observations detected higher ROS status in the SVZ of postnatal mice, which is probably related to a proliferative characteristic of NPCs within the neurogenic niche [7]. Similarly, *in vitro* studies using different models of neural development, including embryonic stem cells (ESCs), teratocarcinoma stem cells and neuroblastoma cells, point ROS as a key factor in neural differentiation, while the impairment of ROS formation prevents differentiation (reviewed by [14]). In addition, more recently, Le Belle and colleagues [7] described differences between NPCs derived from human ESCs (hESCs): a sorted population characterized by high (ROS^{hi}) and another one, with lower levels of ROS (ROS^{lo}). They showed that ROS^{hi} population has a greater proliferative and self-renewal capacity when compared to ROS^{lo} or unselected cells. Accordingly, unselected NPCs acquired the same characteristics as the ROS^{hi} population when exogenous ROS was administered *in vitro*. Moreover, after sorting primary adult SVZ cells through sets of neural stem cell markers, the authors identified that this population presented also significantly high endogenous ROS levels, which indicate that the ROS^{hi} population is composed of neural stem cells. Finally, they showed that ROS generation in NPCs could be induced by specific growth factors, such as brain-derived neurotrophic factor (BDNF), and that it is highly dependent on PI3 k/Akt signaling and NADPH oxidase (NOX) [7]. Thus, low-O_2 levels and high-ROS levels could be associated to a proliferative state of NPCs. However, within the neurogenic niche, a portion of the stem cells remains quiescent and only divides when they receive some stimuli. Those cells are expected to maintain a lower level of ROS until they are required to divide.

Due to the importance of O_2/ROS signaling in the formation of the nervous system, its impairment or modulation may contribute to abnormal brain development that can be perpetuated into adult life. Several neurodevelopmental disorders were related to altered energy metabolism and/or oxidative stress during early development [17,18]. Moreover, a large number of genetic and environmental factors considered as risk factors for these disorders are known to alter O_2/ROS levels or interfere with ROS-mediated signaling. Thus, understanding the role of O_2/ROS signaling during neurogenesis will help to identify novel aspects of neurodevelopmental disorders, such as schizophrenia.

Schizophrenia

Schizophrenia is a mental disorder that affects one in a hundred individuals [19]. In spite of many efforts to define general characteristics of this disorder, schizophrenia is a very heterogeneous syndrome and manifests itself quite peculiarly in each individual [20]. Nevertheless it can be characterized by three types of symptoms: (i) positive symptoms (hallucinations, delusions and abnormal thinking), (ii) negative symptoms (apathy, difficulty in expressing emotions and poverty of thought), and (iii) cognitive deficits (affecting attention, working memory, learning, problem solving and social cognition).

In contrast to the incidence of the disorder (1%), this number could reach 17% for close relatives of a patient [19], suggesting the involvement of a strong genetic component. A great variety of genes have been already correlated to increased risk to schizophrenia outcome [21], and some of them are suggested to be influenced by O_2/ROS levels as discussed below [6]. On the other hand, the co-occurrence of schizophrenia in identical twins happens in almost 50% of the cases [22], indicating that environmental factors are also related to its pathogenesis.

Infectious insults and toxic or traumatic stress during pregnancy or childhood are some examples of environmental factors that were already identified to have an important role in the pathogenesis of schizophrenia through subtle changes in nervous system development [18]. Most of all factors that have been related somehow with the etiology of schizophrenia, including genetic and environmental ones, are revealed during the maturation of developing brain circuits. Accordingly, there are several evidences suggesting that schizophrenia is indeed a neurodevelopmental disorder [20], in spite of being usually diagnosed in the adolescence [23,24].

The identification of pathophysiological changes related to schizophrenia is essential to better understand cellular aspects of the disorder. Unlike other diseases that affect the nervous system such as Parkinson and Alzheimer, there are not many reports linking schizophrenia to progressive neurodegeneration or cell death, instead, it is best characterized by changes in GABAergic, dopaminergic, serotonergic and glutamatergic brain circuits [25]. Recently, alterations in ROS balance and metabolism have also been associated to schizophrenia [17].

The main challenge is to correlate cellular phenotypes *in vitro* with the symptoms of the disorder, in order to facilitate translational studies and also high-throughput screening (HTS) of new drugs.

Induced pluripotent stem cells as a new model to study neurodevelopmental disorders

Due to difficulties in obtaining live neurons from schizophrenic patients, most of the knowledge about this mental disorder has been acquired with brain imaging or *postmortem* neural tissue [26]. As the latter represents the final stage of the disorder, information could frequently be a secondary effect, such as a consequence of chronic treatment with a medicine or even aging [27]. Finally, *postmortem* brains are not elusive for a causative description of neurodevelopmental disorders [28].

In order to overcome these limitations, research has been done in non-neural cells (e.g. blood cells); however, it is still not determined whether studies with these cells are informative enough regarding clinical aspects of schizophrenia [29].