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Review

Adrenomedullary progenitor cells: Isolation and characterization of a multi-potent progenitor cell population

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ABSTRACT

The adrenal is a highly plastic organ with the ability to adjust to physiological needs by adapting hormone production but also by generating and regenerating both adrenocortical and adrenomedullary tissue. It is now apparent that many adult tissues maintain stem and progenitor cells that contribute to their maintenance and adaptation. Research from the last years has proven the existence of stem and progenitor cells also in the adult adrenal medulla throughout life. These cells maintain some neural crest properties and have the potential to differentiate to the endocrine and neural lineages. In this article, we discuss the evidence for the existence of adrenomedullary multi potent progenitor cells, their isolation and characterization, their differentiation potential as well as their clinical potential in transplantation therapies but also in pathophysiology.

Contents

1. Introduction

The adrenal gland is composed of two endocrine tissues of different embryologic origin, the mesodermally derived adrenal cortex and the neural crest derived chromaffin cells of the adrenal medulla. The adrenal is thus particularly suitable to study tissue development, the tissue microenvironment and interactions of different cell types during development and adaptation to physiological needs. We previously demonstrated the critical role of cellular interactions of both steroid and catecholamine producing cells in adrenal physiology and disease (Bornstein et al., 2008; Ehrhart-Bornstein et al., 1998). Furthermore, both adrenal cortex and medulla are highly plastic and able to adapt to physiological needs, suggesting the involvement of progenitor cells. Intensive work within the last years

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has identified the involvement of adrenocortical stem cells in the regeneration and adaptation of the adult adrenal cortex (references in this issue; Walczak and Hammer, 2015). Little, however, is known on progenitor cells in the adrenal medulla, their proliferation and differentiation.

2. Sympathoadrenal progenitor cells in the adrenal medulla

2.1. Are sympathoadrenal progenitor cells maintained in the adult adrenal medulla?

Chromaffin cells of the adrenal medulla develop from neural crest stem cells. Stem cells are defined as an entity with unlimited self-renewal and the capacity to give rise to multiple cell types (Seaberg and van der Kooy, 2003; Weiner, 2008) while progenitor cells possess a limited self-renewal capacity and are often uni- or multipotent. Together with sympathetic neurons of the dorsal ganglia and the intermediate small intensely fluorescent (SIF) cells chromaffin cells develop from a common sympathoadrenal progenitor (Shtukmaster et al., 2013). Along their migratory route, sympathoadrenal progenitors become catecholaminergic, aggregate at the dorsal aorta, and then further migrate to the adrenal anlagen, where they differentiate into mature chromaffin cells (reviewed in Huber et al., 2009).

It is now apparent that various adult tissues maintain neural crest-derived multipotent progenitor cells (for example: Brandl et al., 2009; Davies et al., 2010; Sieber-Blum and Hu, 2008; Widera et al., 2009). Multipotent progenitor cells are often considered adult stem cells. This, however, neglects the functional properties of progenitor cells gained during differentiation that render them distinct from stem cells. Already 30 years ago, SIF cells, the third cell type in the sympathoadrenal branch of the neural crest lineage which exist primarily as a minority cell population in autonomic ganglia, were suggested to constitute sympathoadrenal progenitors that persist in the adult and retain their stem cell properties. These cells exhibit a progenitor phenotype and progenitor properties reflected by their potential to differentiate to sympathetic neurons and chromaffin cells (Doupe et al., 1985a, 1985b). If neural crest-derived sympathoadrenal progenitors are maintained in adult tissues such as ganglia, these progenitor cells might also persist in the adult adrenal medulla where they could contribute to the gland's adaptation to physiological needs.

Work in recent years from our group indeed proved the existence of sympathoadrenal progenitor cells in the adult adrenal medulla. Sympathoadrenal and neural progenitors share some properties in terms of gene expression patterns, lower self-renewal throughout life and their ability to differentiate into functional neurons *in vitro*. Based on this, cells with progenitor properties were first identified in and enriched from bovine adrenal medulla (Chung et al., 2009). Similar to neural stem cells, these progenitor cells, when prevented from adherence to the culture dish, grow in suspension as free-floating spheres with self-renewing capacity, which we named chromospheres. Importantly, sympathoadrenal progenitor cells could also be identified in the adult human adrenal medulla and cultured *in vitro* (Santana et al., 2012). The progenitor cells from adrenal medulla which were enriched in chromospheres share significant properties with neural stem cells, expressing specific stem cell markers including neural stem cell markers such as nestin, CD133, Notch1, nerve growth factor receptor, musashi1, Snai2, Sox9 and Sox10 (Chung et al., 2009; Santana et al., 2012; Vukicevic et al., 2012b) and proteins of the Notch pathway (Vukicevic et al., 2012a). Sox9 and Sox10 are members of the SoxE subgroup, which play an important role in the migration and differentiation of neural crest derivatives (Cheung and Briscoe, 2003; Cheung et al., 2005; Kim et al., 2003) and the specification and survival of chromaffin precursor cells during embryonic development (Reiprich et al., 2008). Due to the fact that Sox10 expression is down-regulated in the adult adrenal medulla, its importance was thought to be restricted to adrenal medulla development (Reiprich et al., 2008). Our data, specifically the expression of SoxE genes in the adult adrenal medulla, however, suggest the persistence of neural crest derived progenitor cells.

2.2. Isolation and characterization of sympathoadrenal progenitors

The isolation of sympathoadrenal progenitor cells is one important goal in order to study their properties in a homogenous culture. Several flow cytometry methods were used to identify and isolate stem cells based on metabolic activity. The expression of high levels of aldehyde dehydrogenase (ALDH) activity and staining with a fluorescent substrate for ALDH has first been described to identify and isolate primitive hematopoietic stem cells (Fallon et al., 2003; Jones et al., 1995) and ALDH-bright ($ALDH^{br}$) cell populations with stem cell activity have in recent years been sorted from several normal tissues (Balber, 2011). The ALDH expression assay has also been adapted to effectively identify and isolate neural stem cells (NSCs) and progenitors from adult and embryonic murine neurospheres and dissociated tissue (Corti et al., 2006; Obermair et al., 2010).

Based on these previous reports, we assumed that medullary sympathoadrenal progenitor cells might contain high levels of ALDH that could be used as a novel identification marker for their isolation and characterization. The fraction of sympathoadrenal progenitor cells was enriched from chromospheres based on the intracellular ALDH activity using the ALDEFLUOR (StemCell Technologies, Köln, Germany) method (Balber, 2011). The progenitor population was detected and isolated by flow cytometry (FACSAria, Becton-Dickinson, Franklin Lakes, NJ, USA) due to their pronounced activity of ALDH resulting in higher intensity of fluorescence ($ALDH^{br}$) and characteristic low granularity displayed as Side Scatter Low ($ALDH^{br}SSC^{lo}$) (Fig. 1).

To investigate the gene expression profile in this potential progenitor cell population, a microarray analysis of gene expression was performed in isolated progenitors ($ALDH^{br}SSC^{lo}$) sorted compared with $ALDH^{low}$ chromosphere cells (Fig. 2). On the one hand, several genes characteristic of neural or neural crest progenitor and stem cells were detected in the $ALDH^{br}SSC^{lo}$ population. Up-regulation of Notch2 and its downstream effector Hes1 has been described in neural stem cells (Borghese et al., 2010; Solecki et al., 2001), preventing differentiation and promoting self-renewal. The expression in the $ALDH^{br}SSC^{lo}$ population suggests a similar function in sympathoadrenal progenitor cells. This is in accordance with our previous data showing Notch2 and Hes1 expression in chromosphere cells (Vukicevic et al., 2012b). The expression of genes involved in other pathways indicates diversity of molecular mechanisms in the regulation of sympathoadrenal progenitor cells. This for example includes Wnt2B ligand that, as a part of the canonical pathway, plays an important role in the regulation of neural crest progenitor cell differentiation during eye development (Grocott et al., 2011; Kubo et al., 2005). Upregulation of WLS and WISP2 genes is considered to contribute to the control of stem cell renewal downstream of Wnt (Wend et al., 2010). Moreover, isolated sympathoadrenal progenitors displayed upregulated homebox transcriptional repressor MSX1 which is involved in the regulation of neural crest specification and is particularly expressed in neural ectoderm (Gammill and Bronner-Fraser, 2003; Ishii et al., 2005).

On the other hand, genes characteristic for differentiating or differentiated neural and/or chromaffin cells were downregulated. These include alpha and beta tubulins involved in neural differentiation. Moreover, genes involved in the differentiation of sympathoadrenal progenitors such as ASCL1 (MASH1) (Huber et al., 2002), GATA2 (Tsarovina et al., 2004) and HAND1 (Vincentz et al., 2012) and genes involved in catecholamine synthesis such as