Molecular and Cellular Endocrinology 445 (2017) 1–6

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

Future perspectives in adult stem cell turnover: Implications for endocrine physiology and disease

Many organs and tissues are now considered to make up the endocrine system, amongst them entire epithelial glands, parts of otherwise non-endocrine organs and also non-glandular tissues such as skeletal muscle and white adipose tissue. In every case, the endocrine cells age or become altered and are renewed by cell turnover. Postnatal endocrine adult stem cells (ASC) have recently been found to underpin cell renewal in many but not all endocrine organs. During the last years, the number of ASC in a given organ has also been associated with the incidence of cancer in that organ (Tomasetti and Vogelstein, 2015).

In this Special Issue, current understanding of ASC is reviewed for several endocrine organs, including the hypothalamus, pituitary, pancreas, adrenal, ovary, testis, skeletal muscle and white adipose tissue. Evidence for the recent concept of endocrine ASC alteration to form cancer stem cells (CSC) able to induce tumours is also reviewed. Finally, the discovery of embryonic stem cells (ESC) and, more recently, artificial pluripotent stem cells (iPS) has led to much work to generate protocols to direct such cells to specific fates, which is reviewed in relation to obtaining differentiated thyroid cells from ESC and its future applications.

During the last few years key discoveries have been also made in the stem field in non-endocrine organs. In this initial Overview, with the aim of opening up new avenues in endocrine stem-cell research, important breakthroughs made recently in non-endocrine organs are reviewed. Only work from the last three years is revised here and specifically anything related to the organs protagonists of the following reviews in this Special Issue in Stem Cells in Endocrine Organs is not included.

1. Recent innovations to overcome technical limitations in stem cell research

Working with ASC is inherently challenging due to their small, poorly defined and widely dispersed populations. Although more than one such population usually exists in any organ, the molecular taxonomy of ASC is poorly defined and probably differs from one organ to another. Cells can lose their stem properties and behave anomalously in vitro after isolation from their in vivo niche. For ESC and iPS there is an additional challenge, to certify at the molecular level “differentiation” to the required cell type.

In recent years some interesting technological advances have overcome some of these challenges, the most relevant being:

1.1. Single-cell RNAseq (scRNAseq)

Improvements in high-throughput technologies in nucleic acid amplification and sequencing have made it possible to sequence the RNA of a single cell (Wilson et al., 2015; Treutlein et al., 2016). While this is not a very sensitive method by which to quantify all RNAs expressed in a cell, it is a robust technology for identifying subsets of co-expressed genes that characterise different populations coexisting in an organ. Mathematical algorithms such as Monocle, Seurat, Waterfall, Sincera, Giniclust (Trapnell et al., 2014; Satija et al., 2015; Guo et al., 2015) are also implemented to define such populations in the organ as a whole, and data collated in public databases such as CODEX (Sanchez-Castillo et al., 2015).

1.2. In vivo microscopy

Knowledge about ASC has been obtained from cell culture experiments using sorted populations and sections of fixed organs in genetic tracing mouse models, in control or experimental animals. With the emergence of two-photon microscopy and its application to live animals it has also been possible to test the accuracy of data from fixed tissues by comparing with living tissues (Barbosa et al., 2015; Rompolas et al., 2016). As commented below, in vivo, ASC from solid tissues appear to function differentially from hematopoietic ASC, while mechanisms of cell turnover might be different in different organs.

1.3. Organoids

Primary culture in 2D is an established method to study the behaviour and differentiation of ASC. More recently, development of 3D culture systems has demonstrated that a single ASC can generate structures resembling crypts and villus, in the case of a smooth intestinal epithelial ASC (Sato et al., 2009). Now it has been extended to oesophagus, human colon and pancreas, mouse neural tube and primate brain from appropriate ASC (DeWard et al., 2014; Matano et al., 2015; Huang et al., 2015; Ranga et al., 2016; Otani et al., 2016). 3D culture, moreover, allows manipulation of both the environment and the genetic background of the initial ASC to analyse generation of functional structures.

http://dx.doi.org/10.1016/j.mce.2016.12.008

1.4. CRISPR/Cas9 editing of stem cells

Sequence-specific DNA editing is considered the technological highlight of biological research in the last five years (Jinek et al., 2012). The technology is currently being applied to differentiation of iPS, both to generate models of disease and to engineer potential cures for genetic diseases (reviewed in Hockemeyer and Jaenisch, 2016).

2. Dynamic heterogeneity of ASC: many organs and as many different ASC

2.1. ASC cell identity: markers, markers!!

As commented above, many organs including endocrine glands have been found to have more than one cell population expressing stem cell markers. For example, studies in human, mouse and rat pituitary demonstrate that within the stem-cell niche, quiescent ASC co-express several markers at high levels (e,g, Gfra2, Ret, Sox2, Sox9, Oct4, Klf4, beta-Catenin) (Garcia-Lavandeira et al., 2009, 2012). However, other pituitary cell populations are positive for only one stem marker without co-expression of the others (Garcia-Lavandeira et al., 2015). It is assumed, but has not been convincingly demonstrated, that the single-positive cells are derived by further commitment of co-expressing cells, as part of a progressive transition to differentiation.

Genetic animal models of cell tracing are based on a single gene, e.g. Sox2, and the fluorescent tracer will remain even when the cell stops expressing Sox2. The label therefore cannot distinguish ASC co-expressing stem markers from those that are single-positive for the targeted marker such as Sox2 in this case. This problem affects the study of ASC in many organs, at times generating apparently conflicting results, although in reality the data might be derived from single-positive versus co-expressing cell populations.

Current consensus is that, at least in epithelial organs with a high cell turnover such as skin or intestine, heterogeneous populations of stem cells co-exist and are highly dynamic (Donati and Watt, 2015; Clevers, 2015). In this context, the Confetti model – in which one of many possible loxP-Stop sites is recombined per cell, labeling each ASC with a different fluorescent protein (Wu et al., 2016)-, has showed that some clones derived from a single ASC are long lived and give rise to the full range of differentiated cell type derivatives, while other ASC disappear immediately as a result of differentiation (Donati and Watt, 2015). And importantly, it seems that many, if not all, of these different populations are able to behave as multipotent cells in certain conditions such as in response to severe injury or when isolated in vitro (Baggiolini et al., 2015). The demonstration that one ASC population is able to generate all types of differentiated cells in a given tissue does not exclude the possibility that ASC from another population can do so too. Thus, homeostasis seems to be maintained in many solid organs through a dynamic heterogeneity of ASC (Greulich and Simons, 2016). But the main rule for one fate (retaining stemness) versus the other (differentiating) seems to be “chance”.

2.2. Cell and tissue turnover: hierarchical versus stochastic models

Hematopoietic ASC are the best studied ASC, being the first to be discovered and relatively easy to isolate and purify for in vitro and transplantation experiments. As a result of many such studies a model has arisen whereby the majority of pluripotent ASC are quiescent but able to be activated and to divide. This division can be either symmetric, giving rise to two pluripotent ASC, or asymmetric, giving rise to one pluripotent ASC plus one restricted progenitor with less potency that generates different lineages. Through a series of successive symmetric and asymmetric divisions of those progenitors, all the range of hematopoietic lineages is generated both in diversity and in the required quantities. This Hierarchical Model is widely accepted and had been proposed as the norm for all organs with ASC niches (Fig. 1A).

However, this model seems hard to reconcile with data obtained in solid organs using genetic animal models of stem-cell tracing. Specifically, it seems that asymmetric division occurs more readily in vitro than in vivo. This year, using in vivo microscopy, it was possible to label a few epidermal stem cells with a fluorescent protein and to follow their fate in vivo (Rompolas et al., 2016). The data showed that in the skin an epidermal ASC had a 50% chance of dividing symmetrically to generate two ASC or to differentiate directly without dividing. Moreover, no regulated asymmetrical cell divisions were observed. This indicates a Stochastic Model of cell turnover in epidermis, and probably for many solid organs (Fig. 1B).

Future work will show which model better reflects cell turnover in different endocrine organs. However, at least for the pituitary, the idea of direct – as opposed to progressive – differentiation does not fit with the existence of various stem populations outside the niche of quiescent ASC and single-positive for one stem marker (Garcia-Lavandeira et al., 2009, 2012 2015; Alvarez et al., 2012). The cells in these populations cannot be considered to be ASC (being outside the niche and showing no co-expression of stem markers), and yet they do not express hormones or transcription factors required to consider them as differentiated endocrine cells. Thus, for a solid organ it could be that cell turnover follows a ‘progressive’ model in which some hierarchy is maintained during commitment and differentiation. In this sense, in the airway epithelium a signal, in the form of a secreted Notch ligand, is constantly secreted from the basal stem cells in a forward manner towards committed secretory cells and terminally differentiated ciliated cells (Pardo-Saganta et al., 2015). Alteration of Notch signaling leads to sudden differentiation of all committed secretory cells, reducing amplification and compromising cell turnover in the long term.

On the other hand, some glands such as the endocrine pancreas lack a clear ASC niche and might be maintained through transdifferentiation. There are, moreover, species-specific differences in cell turnover of any given organ, as demonstrated by neural ASC: primate neural progenitors proliferate over an extended period of time, whereas rodent neural progenitors do not (Otani et al., 2016), with the end result being a difference in the number of neurons generated.

2.3. Physiological renewal versus repair after injury

Another important factor in understanding complexity of ASC and explaining contradictory results is the model in which data are obtained. From studies in the skin, intestine and bone marrow it has become apparent that the mechanism of physiological cell turnover is quite different from that of repair after injury (Donati and Watt, 2015; Clevers, 2015).

Many reports of multipotent ASC populations have been based on implantation of a single ASC in either radiation-depleted bone marrow, injured skin or following genetic injury by diphtheria toxin in differentiated cells. It now seems that under conditions of stress or inflammation cells can regress through differentiation, recovering multipotency and the ability to mitose (Fig.1B). In some cases, such conditions can induce non-stem-cell related mechanisms of repair, such as proliferation of previously quiescent differentiated cells following hepatectomy, or direct transdifferentiation after massive beta-cell loss in the pancreas (Barbosa et al., 2015) (Fig. 1B). Thus while understanding repair or regeneration in response to acute cell loss through injury, surgery or auto-