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Regenerative therapy for hypothyroidism: Mechanisms and possibilities

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ABSTRACT

The ability to derive functional thyroid follicular cells from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) would provide potential therapeutic benefit for patients with congenital or post-surgical hypothyroidism. Furthermore, understanding the process by which thyroid follicular cells develop will also provide great insight into the key steps that regulate the development of other tissues derived from endoderm. Here we review the advances in our understanding of the process of thyroid follicular cell development including the creation of two models that have allowed for the rescue of hypothyroid mouse recipients through the transplantation of thyroid follicular cells derived from mouse ESCs. Rapid progress in the field suggests that the same success should be achievable with human ESCs or iPSCs in the near future. Additionally, the availability of ESC or iPSC-derived thyroid follicular cell models will provide ideal systems to explore how genetic mutations, drugs or illness impact thyroid function in a cell-autonomous fashion.

1. Introduction

The development of novel modalities of thyroid hormone replacement have been considered for years given the issues with clinical efficacy of L-thyroxine replacement. Indeed, it is now clear that a significant number of patients requiring thyroid hormone replacement therapy do not feel well on replacement therapy. With the advent of stem cell technologies it is now possible to envision cellular replacement therapies for those with either congenital or post-surgical hypothyroidism who are dependent on exogenous replacement therapy. In contrast regenerative therapy may be difficult in patients with autoimmune thyroid disease given issues with tissue rejection and the likelihood that transplanted cells would be destroyed if autoimmunity is not first addressed. While the clinical merits of exogenous therapy versus the production of endogenous thyroid hormone by a transplanted gland can be debated it is clear that insights gained from a better understanding of follicular cell development may lead to clinical treatments for some that may be preferred over pharmacologic therapy. This is especially the case for children with congenital hypothyroidism whose genetic defects could potentially be corrected ex vivo in their cultured stem cells prior to transplantation of stem-cell derived thyroid follicular cells (De Felice and Di Lauro, 2004; 2011). Additionally, cellular models that recapitulate follicular cell development will be able to provide key insight into developmental milestones as well as provide ex-vivo models to test the role of novel pathways or drugs on thyroid development and function. In the following article we will review the progress made in the development of functioning thyroid follicular cells from embryonic or induced pluripotent stem cells to date.

2. Early thyroid development

The fully developed thyroid gland contains two endocrine cell types. The minority component is made up of parafollicular cells (C cells) which secrete calcitonin and derive from the neural crest. The majority component within the thyroid gland are endodermally derived follicular epithelial cells and the focus of this review. Indeed, as will be discussed, the derivation of fully functioning thyroid tissue from embryonic stem cells in now way requires the presence of calcitonin-secreting C cells.

Beginning at E20 in humans and E8.5 in mice, the follicular cell development program begins in the anterior foregut endoderm as a small subset of endodermal progenitor cells commit to a thyroid

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cell fate through a process referred to as "lineage specification". Follicular cell fate is defined by the co-expression of the transcription factors Nkx 2-1 and Pax8, the earliest known markers of thyroid lineage specification within developing endoderm (Carre et al., 2011; Davies et al., 2011; Lazzaro et al., 1991; Parlato et al., 2004). Indeed, while these transcription factors are expressed in other cell-types it is their co-expression at a unique developmental stage that first identifies the developing endodermal thyroid primordium and is required for normal, subsequent follicular cell development (Damante et al., 2001; Kimura et al., 1996; Mansouri et al., 1998). What extrinsic factors cause the initial thyroid cell fate decision have not been completely defined but a role for factors released by the adjacent cardiac mesoderm has been implicated by some studies (Celli et al., 1998; Fagman et al., 2007; Fagman and Nilsson, 2010; Lania et al., 2009; Vitelli et al., 2002; Kameda et al., 2009) and our recent work suggests combinatorial BMP and FGF signaling as two likely pathways that induce thyroid fate in the developing foregut endoderm of xenopus, mice, and humans. Close to the time of this cell fate decision additional transcription factors including Foxe1 and Hhex are expressed in the thyroid primordium, which together with Nkx2-1 and Pax8 form a core network of transcriptional regulators leading to differentiation, migration and maturation of the developing follicular cells (De Felice and Di Lauro, 2011; Parlato et al., 2004). The importance of these transcription factors is further underscored by the fact that mutations in Nkx2-1, Pax8 and Foxe1 lead to congenital hypothyroidism in humans (Krude et al., 2002; Trueba et al., 2005; Castanet et al., 2002). In addition to these key transcription factors, roles for the Hox genes and Eya1 gene have been implicated in thyroid development (Manley and Capecchi, 1995, 1998; Xu et al., 2002). While growth factors that induce FGF and BMP signaling appear to regulate the thyroid cell fate decision TSH which is a major growth factor for the gland is not required for lineage specification but does play a role in thyroid gland growth and function (Kurmann et al., 2015). Mice lacking a functional TSH receptor are hypothyroid but possess a hypoplastic gland that contains a normal structure (Beamer et al., 1981; Marians et al., 2002; Postiglione et al., 2002). A similar phenotype is seen in humans with inactivating TSH receptor mutations (Abramowicz et al., 1997; Biebermann et al., 1997). Given the understanding of the transcriptional pathways employed in follicular cell development it became logical to try and re-create follicular cell development by activating these genes or pathways in vitro in cell-based models.

3. Thyroid function in embryonic stem cells – initial studies

Since the discovery of the pluripotency of embryonic stem cells (ESCs) there has been much focus on the derivation of specific cell types for potential therapeutic application. In the thyroid field initial studies focused on whether ESC-derived embryoid bodies, which contain the three germ layers of the embryo could express thyroid follicular genes. Lin *et* al developed embryoid bodies from a mouse ESC line which in the undifferentiated state expressed no follicular cell markers (Lin et al., 2003). After 6 days in culture they began to see the expression of the sodium iodide symporter (NIS), the thyrotropin stimulating hormone receptor (TSHr) and Pax8 which persisted in culture. They were able to validate TSHr expression using immunohistochemistry and subsequently by demonstrating an increase in cAMP production in response to recombinant TSH and an enhancement in Pax8, thyroid peroxidase (Tpo) and NIS gene expression (Fig. 1). Although these early cells did not express thyroglobulin (Tg), a developmental marker of thyroid maturation known to occur earlier than Tpo or NIS *in vivo*, still this proof of concept study demonstrated that differentiating ESCs had the capability to express both thyroid follicular specific transcription factors and a subset of the genes required for thyroid hormone synthesis.

To follow-up on this work this same group next used an ES cell line that expressed green fluorescent protein (GFP) under the control of the TSHr genomic locus on one allele ($TSHr^{GFP}$) (Arufe et al., 2006). These same ES cells had been used to generate a TSHr knockout mouse which had severe congenital hypothyroidism which was fatal by 4 weeks of life (Marians et al., 2002). Importantly, though hypothyroid these animals developed a hypoplastic thyroid with some thyroid follicles demonstrating that neither thyroid lineage specification nor initial follicular cell development requires TSH signaling. Importantly, these genetically altered ESCs functioned similarly in the undifferentiated state as wild-type ES cells and when differentiated into embryoid bodies upregulated both GFP and TSHr production indicating that the expression of GFP could be used as a marker of TSHr transcription. Next these investigators sorted day 4 $TSHr^{GFP}$ positive cells and cultured them in TSH and Matrigel to allow for a 3-dimensional structure to occur (Martin et al., 1993). Although the sorted cells and their progeny did not express thyroglobulin, after 21 days in culture these cells appeared to form thyroid follicle-like structures and demonstrated by immunohistochemistry correct spatial localization of both NIS and the TSHr (Fig. 1). Furthermore, when cultured in the presence of TSH these cells could take up ^{125} I and this process was specifically inhibited by perchlorate. Finally, there was extensive induction in these cultured cells of Pax8, NIS, Tpo and the TSHr when compared to undifferentiated cells. Thus, further enhancement of the differentiation process was obtained by sorting the cells based on $TSHr^{GFP}$ expression as well as the use of Matrigel and TSH. Still the surprisingly rapid in vitro expression of TSHr, a marker that is not expressed *in vivo* until late in thyroid development, and the absence of Tg expression in this system, highlighted the ongoing challenges facing investigators attempting to achieve full follicular cell development from stem cells in vitro.

Finally, given that thyroid follicular cells could develop in the absence of a TSH signal, these investigators set out to understand what pathways delineated follicular cell development prior to the onset of TSH-signaling. To do this Ma et al. employed activin A a known inducer of endoderm to determine if follicular cell development could be enhanced (Kubo et al., 2004). Activin A was added to embryoid bodies and as expected caused a dramatic upregulation of endodermal markers such as Gata4 and FoxA2. However, the addition of TSH and IGF-1 to Activin A did not enhance the appearance of endoderm (Arufe et al., 2009). Exposure of cells to 5 days of Activin A allowed for the enhanced detection of NIS in $TSHr^{GFP}$ positive cells even though TSHr expression was low and Tg was absent. After more prolonged exposure to Activin A increases in Pax8 was also seen. Interestingly, synergy between Activin A and TSH was not evident but the developed cells did respond to TSH as evidenced by cAMP production. These experiments confirmed the notion that endodermal development of follicular cells could occur independently of a TSH signal, but the exact paradigm for differentiating ESCs into endoderm competent to express all the markers or differentiation kinetics typical of developing thyroid follicular tissue (e.g. foregut endodermal Nkx2-1 and Pax8 expression prior to Tg or TSHr) was not yet established.

4. Follicular cells can be derived from overexpression of Nkx2-1 and Pax8

Because the approach was still unknown for differentiating ESCs into thyroid follicular cells via endoderm, the Costagliola group set out to determine if co-expression of Nkx2-1 and Pax8 could allow for follicular cells to develop from ESCs without needing to first optimize their differentiation into anterior foregut endoderm