REVIEW

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Thymic epithelial progenitor cells and thymus regeneration: an update

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The thymus provides the essential microenvironment for T-cell development and maturation. Thymic epithelial cells (TECs), which are composed of thymic cortical epithelial cells (cTECs) and thymic medullary epithelial cells (mTECs), have been well documented to be critical for these tightly regulated processes. It has long been controversial whether the common progenitor cells of TECs could give rise to both cTECs and mTECs. Great progress has been made to characterize the common TEC progenitor cells in recent years. We herein discuss the sole origin paradigm with regard to TEC differentiation as well as these progenitor cells in thymus regeneration.

Introduction

In general, stem cells are characterized by two fundamental properties of self-renewal potential and further differentiation into several specialized cell types. Stem cells, especially tissue- or organ-specific stem cells obtained from adults, which hold great clinical potential, have attracted much attention recently [1-3]. The tissue stem cells or somatic stem cells from numerous adult tissues, with striking potential ability to repair damaged tissues and even to differentiate into other tissue and cell types, have been identified in both animals and humans [4-6]. Tissue-specific stem cells generally undergo asymmetric division, which thus gives rise to two different kinds of daughter cells. One population continues to maintain the multipotent potential whereas the secondary population becomes specialized cells responsible for the function of the tissue or organ.

It has been well defined that the thymus provides an optimal and essential microenvironment for T-cell development and maturation, although the exact molecular signal-ling events still remain poorly understood [7-9]. Only the thymocytes recognizing major histocompatibility complex (MHC)-self peptide complexes in an appropriate avidity can survive the stringent positive and negative selections and then become functionally and phenotypically mature [10, 11]. This process generally involves extensive interactions between developing thymocytes and thymic stromal cells [12-17]. The thymic stromal cells may provide the growth factors, cytokines and extracellular matrix for T-cell development [18], among which thymic epithelial cells (TECs) form the basic three-dimensional architecture that has the ability to facilitate T-cell differentiation and maturation from the common lymphoid progenitors. The proper differentiation and organization of different TECs is pivotal for both thymocyte development and T-cell repertoire selection. Moreover, TECs are divided into two major categories: thymic cortical epithelial cells (cTECs) and thymic medullary epithelial cells (mTECs), which have been demonstrated to participate in positive and negative selection, respectively [12]. In addition, thymic non-epithelial stromal cells have also been suggested to play important roles at various stages of T-cell development [17, 19].

Homeostasis of epithelial tissues is usually maintained by continuous self-renewal of epithelial stem cells. Although it remains controversial whether the common stem

cells for both cTECs and mTECs reside in the thymus, an emerging body of data support the existence of thymic epithelial progenitor/stem cells (TEPCs/TESCs) [20-24]. Significant progress has been made to identify and characterize TEPCs using lineage track analysis and clonal assays in recent years. In this manuscript, we will summarize data concerning the identification, characterization and purification of TEPCs as well as the potential therapeutic application of TEPCs in thymus regeneration.

Thymus organogenesis

Now, there are at least two strategies to study early thymus organogenesis in the mouse: fluorescent cell tracker and ectopic grafting technique for *in vitro* and *in vivo* analysis, respectively [25]. The former mainly focuses on characterizing the location of a distinct population of cells in the developing thymus *in vitro* by labelling them with a fluorescent cell tracker dye. On the other hand, ectopic grafting technique is used to test the contribution of the isolated thymic tissue in thymus organogenesis. It is commonly accepted that the thymus and parathyroids arise from a common embryonic region that develops from the third pharyngeal pouch [26]. The thymic rudiment is first visible at about embryonic day 10.5 (E10.5), and it has been suggested that all the three germ layers contribute to the thymus organogenesis. This process is generally divided into two stages: the early stage involves epithelial-mesenchymal interactions in the absence of thymocytes whereas there are mutual interactions between epithelial cells and developing thymocytes at the later stage [27-32]. Epithelial-mesenchymal interactions have been implicated during organogenesis, although the initiating signals for thymus organogenesis remain elusive. It has been well documented that mesenchymal cells play critical roles at different stages of thymus organogenesis [19, 32]. Previous reports have suggested that mesenchymal cells may respond to the initiating instructive signals for thymus induction from the endoderm and in turn support the growth and differentiation of the thymic epithelial rudiment. At E10, neural crest-derived mesenchymal cells initiate physical interaction with the third pharyngeal pouch and subsequently establish the thymic primordium. At about E11, the thymic rudiment begins budding and outgrowth, followed by immigration of the lymphocyte precursors, and then the patterning and differentiation are initiated through epithelial-thymocyte interaction within the mesenchymal-derived capsule [27].

Mutant or genetically modified animal models have provided valuable tools to elucidate molecular regulators responsible for thymus organogenesis. Many transcription factors have been demonstrated to be critical for thymus organogenesis and TEC differentiation. Deficiency of either the paired box family transcription factors Pax1 or Pax9 results in the failure of thymus organogenesis [33-36]. In addition, it has been reported that the Hox transcription factor family member, Hoxa3, is expressed by both the thymic epithelial and mesenchymal cells, and mutation of *Hoxa3* leads to athymia [37]. The forkhead transcription factor FoxN1 is well known for the famous nude phenotype in mice, rats and humans [38, 39]. Although FoxN1 is expressed by all TECs throughout ontogeny, the TEC differentiation consists of FoxN1-independent and FoxN1-dependent stages. In the absence of FoxN1, the initial steps of thymus organogenesis and the formation of the thymus 'anlage' are not impaired. However, further expansion and differentiation of the epithelial cells are prevented, which leads to non-formation of the normal three-dimensional thymic architecture and impaired ability to attract bone marrow-derived lymphoid progenitors [40]. Furthermore, Wnt proteins have been reported to induce FoxN1 expression in TECs [31, 41]. A better understanding of the earlier signalling cascades for thymus rudiment positioning and patterning [40] and detailed studies on the regulation of FoxN1 expression pattern will provide valuable information on thymus organogenesis and TEC differentiation.

cTEC and mTEC differentiation: the new paradigm

Previous morphological and histological studies have suggested the dual-origin model for thymus organogenesis, describing that the cortical epithelium derives from the surface ectoderm of the third pharyngeal cleft, whereas the medullary epithelium derives from the endoderm [29]. But this prevailing view of the dual-origin model for thymus organogenesis has been challenged recently, as both the cell marker and functional studies support the notion that cTECs and mTECs share the same endodermal origin [42-44]. The excellent evidence for the single endoderm origin is provided by chick-quail chimera experiments demonstrating that only the quail endodermal region can produce the functional mature thymus and support the normal thymopoiesis. The lineage analysis shows no evidence for the ectodermal contribution to the thymic rudiment; on the other hand, the isolated pharyngeal endoderm develops a functional thymus with well-organized cortical and medullary compartments when transplanted ectopically [45]. In this regard, the single endoderm origin of TECs is at present preferentially accepted in place of the dual-origin model [24, 29].

Chimeric mice were established by injecting embryonic stem (ES) cells into blastocysts containing the different MHC to test TEPC activity. Analysis of the 'balanced chi-

meras', with roughly equal contributions of the ES cells and blastocyst, indicated that the medulla comprises individual epithelial 'islets', each arising from a single progenitor [46]. However, whether the cortex compartmentalization can be derived from a single progenitor cell remains to be addressed.

Identification and characteristics of TEPCs

Although the view that both cortical and medullary epithelial cells derived from the endoderm was commonly accepted, it remains controversial whether these two morphologically and functionally distinct types of TECs arise from a common bipotent progenitor. As we know, thymic stromal cells are highly heterogenous [47], and the lack of markers specific for distinct subpopulations has significantly hampered our efforts toward further identification and characterization of these cells. The development of a series of panels of monoclonal antibodies (mAb) against different molecule determinants on distinct stromal cells provides valuable tools for further investigation on the contributions of thymic stroma to TEC development [48, 49]. Cell marker studies have demonstrated that the TEC population is homogeneous at the earlier stage of embryonic development. Cytokeratin5 and cytokeratin8 have been identified as markers of cTECs and mTECs in the adult thymus, respectively [50]. Interestingly, during the embryonic development there is a minor population of TECs coexpressing cytokeratin5 and cytokeratin8, raising the possibility of the existence of a rare progenitor cell that may give rise to both cortical and medullary TECs (Figure 1). Several lines of evidence further support the presence of the common precursors by showing that all the epithelial cells of the third pharyngeal pouch at E10.5 express both 4F1 and IVC4 which mark the cTECs and mTECs, respectively [24].

In keeping with previous studies, Gill et al. and Bennett et al. [22, 23] identified a special population of TECs recognized by the MTS24 and MTS20 mAbs. The tiny population of the MTS20+MTS24+ cells expresses both cytokeratin5 and cytokeratin8, consistent with the existence of the potential common progenitors for cTECs and mTECs. Moreover, the putative progenitor marker MTS24 is expressed by almost all cytokeratin-positive cells at E12 [21]. To our excitement, the MTS20+MTS24+ cell population supports the development of both the cortical and medullary compartment characterized by several markers specific for cTECs and mTECs, respectively. Furthermore, they can establish a functional thymus and support T-cell development and maturation when they are re-aggregated in vitro for 24 h and are transplanted into the kidney capsule of nude mice, even by using as
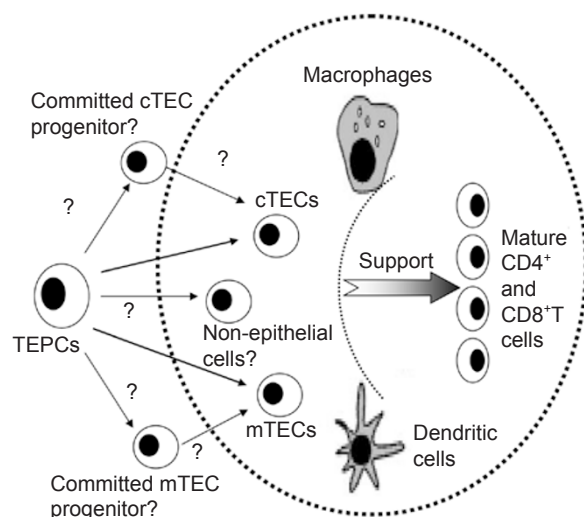
Figure 1 Schematic overview of the precursor-progeny relationship of TECs. The bipotent TEPCs do exist in both fetal and postnatal period, which then give rise to cTECs and mTECs. The TEPCs coexpress markers, which are restricted to adult cTECs and mTECs, respectively. But it remains unclear whether the progenitor cells differentiate into cTECs and mTECs directly or indirectly. cTECs and mTECs, in combination with macrophages, dendritic cells and some other non-epithelial cells, set up the appropriate microenvironment for T-cell development and maturation.

few as 500 MTS20+MTS24+ cells [22, 23]. In contrast, the corresponding MTS20-MTS24- population could not support the apparent cTEC and mTEC development and the peripheral T-cell reconstitution when transplanted in the same manner. Eighty percent of MTS20-MTS24- cells express cytokeratin5 and 10% express cytokeratin8, indicating that some differentiated mTECs and cTECs exist, but it is still unclear why those differentiated cells cannot continue to support T-cell development [23]. Although these studies provide compelling evidence that the potential TEPCs are restricted to the MTS20+MTS24+ cells and that the implantation of MTS20+MTS24+ cells can generate a functional microenvironment for T-cell development, the MTS20+MTS24+ population is heterogenous so that it may contain several types of cells, and thus it remains obscure whether a single stem cell can fulfill functional reconstitution of the thymus. Clonal assay provides the appropriate model to define the differentiation potential of a single cell. Recently, Rossi et al. [21] provided direct evidence that a single progenitor cell can give rise to both cTECs and mTECs as defined by enhanced yellow fluorescent protein (eYFP) expression. Ideal clonal analysis requires a normal microenvironment, which can provide developmental cues

for the stem cell to fulfill their differentiation potential; for this purpose, one YFP-expressing E12 MTS24+ progenitor cell was microinjected into the syngeneic wild-type thymic rudiment controlling the intact three-dimensional structure and then placed under the kidney capsule [21]. Four weeks after the transplantation, YFP expression was observed in both the cortical and medullary compartment as determined by immunofluorescence analysis with several different markers specific for cTECs and mTECs [21].

Now, we know that the MTS20+MTS24+ population has been identified at the embryonic period (either E12.5 or E15.5) [22, 23], but whether the TEPCs exist in the postnatal period remains unclear. Notably, the TEPCs of the thymus rudiment can survive into the adult period in the absence of FoxN1. Thus, using the Cre-Loxp system, Bleul *et al.* [20] studied the function of postnatal TEPCs. Using the well-established Rosa26R-eYFP as a read-out for Cre activity, they elegantly introduced *FoxN1* into the potential TEPCs from the nude mice thymic rudiment. They found that a single epithelial progenitor cell from the *FoxN1*-deficient postnatal thymus when reverted to express the FoxN1, could give rise to a complete and functional thymic microenvironment, and the peripheral T cells were repopulated to some extent in the nude mice, suggesting that cell-based therapies could be developed for thymic disorders . However, it is still unclear whether these cells represent the true adult TEPCs. In this regard, what strikes us most may be the capacity to generate an organ from a small population of cells or even one stem cell. Is it the same case in humans? Are these bipotent TEPCs? Two recent papers published in *Nature* developed novel means to track mammary epithelial stem cells; and an entire functional mammary gland could be regenerated when transplanting only one stem cell ectopically [51-53]. Can we reconstitute functional T-cell development by transplanting a defined population of the TEPCs? If so, TEPC transplantation will greatly benefit older people who have severe thymic atrophy, patients following chemotherapy and radiotherapy, or those infected with the HIV virus. In other words, we may have another chance to build a functional thymus by implanting a few TEPCs [54].

Collectively, several questions regarding TEPCs and their precise role in T-cell development remain to be resolved. One key issue in stem cell therapy is to ensure that the stem cells are placed in the appropriate location to maintain their multipotency, as the niche is critically required for the maintenance of stem cell properties including proliferation and differentiation of stem cells [55-57]. The nature of the TEPC niche and how TEPCs and their supporting cells are nested together remain to be determined. Although it is increasingly obvious that TEPCs contribute to the majority of cTECs and mTECs, we cannot exclude

the possibility that the lineage-committed progenitors for either cTECs or mTECs may also exist (Figure 1). Do those bipotent progenitor cells only differentiate into cTECs and mTECs? The epithelial-mesenchymal transition has been well characterized as the fundamental process in development as well as the major mechanism for tumor metastasis. It remains unclear whether these progenitors could differentiate into several non-epithelial stromal cell types including the dendritic cells and macrophages, which also contribute to T-cell development and T-cell repertoire shaping (Figure 1). However, immunohistochemical analysis of grafting experiments by Rossi *et al.* [21] showed no evidence for the transition of these progenitor cells to other lineages. It is also important to ensure that the transplanted tissue can give rise to a normally developed medullary compartment, which can mediate efficient negative selection and prevent the development of autoimmune diseases, while maintaining the ability to reconstitute the peripheral T-cell compartment. Being able to do so may significantly prompt the use of TEPC transplantation in thymus regeneration.

Concluding remarks and perspectives

Although it is increasingly apparent that a bipotent progenitor cell exists at both the embryonic and postnatal period, further investigations are definitely required to characterize the exact role of MTS24 antigen in the function and development of TEPCs. Are there some differences between embryonic and adult MTS24+ epithelial cells? While ectopic grafting, the E12.5 or E15.5 MTS24+ cells has been proposed to support functional thymus regeneration, so far there is no evidence to document the thymus reconstitution potential of the adult MTS24+ epithelial cells when ectopically transplanted. Thus, it still remains questionable whether MTS24 can serve as a reliable marker to characterize adult TEPCs. In addition, is MTS24 associated with the self-renewal potential or with the proliferation and differentiation of TEPCs? Nevertheless, the increasing knowledge on TEPCs raises hopes for us to achieve thymus regeneration from implanting TEPCs in the future.

Acknowledgments

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