Immunology Letters 269 (2024) 106904

Contents lists available at ScienceDirect

Immunology Letters

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

Single-cell sequencing has revealed a more complex array of thymic epithelial cells

Eleonora Pardini [a,1], Serena Barachini [b,1,*], Greta Alì [c], Gisella Sardo Infirri [a], Irene Sofia Burzi [a], Marina Montali [b], Iacopo Petrini [a]

[a] Department of Translational Research and New Surgical and Medical Technologies, University of Pisa, Pisa, Italy
[b] Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy
[c] Department of Surgical, Medical and Molecular Pathology and Critical Care Medicine, Pisa, Italy

ARTICLE INFO

Keywords:
Thymus
Thymic epithelial tumors
Medullary thymic epithelial cells
AIRE
Cortical thymic epithelial cells

ABSTRACT

Thymic epithelial cells participate in the maturation and selection of T lymphocytes. This review explores recent insights from single-cell sequencing regarding classifying thymic epithelial cells in both normal and neoplastic thymus. Cortical thymic epithelial cells facilitate thymocyte differentiation and contribute to positive selection. Medullary epithelial cells are distinguished by their expression of AIRE. Cells progress from a pre-AIRE state, containing precursors with cortical and medullary characteristics, termed junctional cells. Mature medullary epithelial cells exhibit promiscuous gene expression and after that downregulate AIRE mRNA. Post-AIRE cells can adopt a Hassall corpuscle-like phenotype or exhibit distinctive differentiation characteristics including tuft cells, ionocytes, neuroendocrine cells, and myoid cells.

1. Introduction

The thymus is a primary lymphoid organ specialized in the education of immature T lymphocytes, called thymocytes, to recognize foreign antigens while maintaining tolerance to self-antigens [1].

The thymus is placed into the anterior mediastinum and extends from the lower edge of the thyroid gland to the fourth intercostal space. The human thymus begins developing during the sixth week of embryonic life from the third pharyngeal pouch of the foregut. It reaches its maximum size during puberty and thereafter undergoes atrophy in adulthood. Tissue involution includes loss of thymic epithelial cells (TECs), intensified epithelial-to-mesenchymal transitions leading to an increased number of fibroblasts, fibrosis, and adipogenesis [2]. The thymus is composed of two pyramid-shaped lobes. The thymus is surrounded by a connective tissue capsule, from which septa originate, dividing the parenchyma into lobules. Each lobule is organized into cortical and medullary regions separated by the cortico-medullary junction (Fig. 1). The cortex is the outer region where densely packed immature thymocytes reside, while the medulla represents the inner region with less densely localized mature thymocytes and enriched stromal cells. The thymic stroma is predominantly composed of TECs and additional cell types, including dendritic cells, mesenchymal cells, endothelial cells, and pericytes. TECs are further divided into cortical (cTEC) and medullary (mTEC) based on their physical position in the organ, surface markers, and gene expression patterns (Table 1) [3]. There are functional as well as anatomical differences between the two regions. Each region is composed of different types of cells capable of producing various cytokines and surface molecules to modulate different checkpoints in thymocyte maturation and selection [4]. The major mTEC subset, which appears stellate, exhibits cytokeratin 5 and 14 (KRT5, KRT14) staining. The minor mTEC subset, which appears globular, shows cytokeratin 8 (KRT8) staining. In contrast, the vast majority of cTECs express KRT8 [5]. The cortex stroma encompasses cTECs, thymic nurse cells (TNCs) and macrophages, migratory dendritic cells (DCs), and fibroblasts. The medullary region contains several types of mTECs, macrophages, resident and migratory conventional DCs, plasmacytoid DCs, fibroblasts, and B cells [6]. While cTECs promote T-cell lineage commitment and positive selection, mTECs regulate the elimination of autoreactive T cells with negative selection and regulate the differentiation of regulatory T cells (T_{regs}) [7].

In the cortex, CD4-CD8- double-negative (DN) thymocytes, after interacting with cTECs, initiate their maturation and rearrange the

* Corresponding author at: Department of Clinical and Experimental Medicine, University of Pisa, Via Roma 57, Pisa, 56126, Italy.
E-mail address: serena.barachini@unipi.it (S. Barachini).
[1] Contributed equally as co-first authors.

https://doi.org/10.1016/j.imlet.2024.106904
Received 13 May 2024; Received in revised form 23 July 2024; Accepted 29 July 2024
Available online 6 August 2024

variable regions of their T cell receptor (TCR). Once thymocytes become CD8 + CD4 + double-positive (DP) thymocytes, their TCR can interact with self-peptides presented on the major histocompatibility complex (MHC) class I or II expressed by cTECs. If this interaction occurs with intermediate affinity/avidity, it will lead to positive selection, enabling DP thymocytes to differentiate into CD4 + CD8- or CD4-CD8 + single-positive (SP) thymocytes. DP thymocytes that fail the interaction with antigens presented on the MHC undergo death by neglect in the absence of TCR-mediated survival signals [8]. While DP thymocytes that strongly interact with self-antigens presented by the MHC on the surface of cTEC undergo negative selection in the cortex [9]. After positive selection, the surviving SP thymocytes migrate into the thymic medulla, where they interact with mTECs. In the medulla, thymocytes encounter antigens expressed in our body to gain self-tolerance. Therefore, some mTEC expresses tissue-restricted antigens (TRA) in a process called promiscuous gene expression (PGE) [10]. Thymocytes that express α/β TCR with a high affinity for TRA are eliminated by apoptosis or differentiate in Treg in a process named negative selection, which is essential for central tolerance induction [1]. The intrinsic role of mTECs in expressing TRAs depends on the nuclear factor autoimmune regulator (AIRE) and FEZF2 (forebrain-expressed zinc finger 2, also known as Zfp312 and Fezl) [11,12]. FEZF2 was identified as inducing TRA expression independently of Aire, and both transcription factors are likely to contribute to the generation of TRA repertoires in mTECs [12]. Morphological changes in the thymus become evident by 4 weeks of age in mice, including cortical thinning and the coalescence of medullary regions. These macroscopic changes are paralleled by alterations in both TECs and thymocytes. The total thymic and TEC cellularity halves between 4 and 16 weeks of age [13]. Negative selection declines with the age-related thymic involution with fewer CD4+ and CD8+ SP thymocytes negatively selected in aged mice. The overall reduction in mTEC cellularity and the decline in PGE of TRAs likely diminish the efficiency of antigen presentation, impacting negative selection. Consequently, this leads to an increase in TCR repertoire diversity with age [13].

Both cTECs and mTECs are characterized by a high degree of internal heterogeneity, indicating that they are not homogeneous compartments [14]. The heterogeneity of these populations has recently been elucidated through single-cell sequencing (scRNA-seq) evaluations of murine and human normal thymus. ScRNA-seq has emerged as a powerful tool for uncovering heterogeneity within cellular populations at various levels, including transcriptomic, genomic, and epigenomic [15]. This technique is widely used to reveal changes in disease development, especially in tumors that display diverse phenotypes. Unlike traditional RNA sequencing methods that analyze transcriptomes of mixed cell populations (bulk), scRNA-seq allows for the examination of the transcriptomic profiles of individual cells within rare populations. This enables a more comprehensive understanding of cellular heterogeneity and provides insights into the distinct characteristics of specific cell types within a complex tissue or disease context [16].

In this review, we explore recent advancements in understanding TEC populations in humans and mice and compare the findings with the results of scRNA-seq in thymic epithelial tumors.

2. Murine TEC progenitors

The thymus and parathyroids originate from the ventral and dorsal portion of the endoderm of the third pharyngeal pouch, respectively. In mice, thymus and parathyroid domains are specified by embryonal days (E) 9.5 and E10.5. However, they cannot be morphologically distinguished until E11.5 when Foxn1 and Gcm2 expression begins, respectively. While these transcription factors play a role in regulating differentiation, it's important to note that they do not specifically determine thymus and parathyroid fate [17]. The transcription factors and molecular pathways that regulate thymus fate and organogenesis remain to be fully elucidated. T-box transcription factor (TBX1) plays an indispensable role in thymus development, as individuals with an inactivating mutation or deletion of TBX1 fail to develop a thymus [18]. For example, in the Di George syndrome, deletion 22q11.2 encompasses the *TBX1* locus and can be associated with thymus aplasia or hypoplasia [19]. Moreover, knockout mice with homozygous deletion of *Tbx1* develop thymus aplasia [19]. Even if Tbx1 expression is necessary for the development of the third pharyngeal pouch, Tbx1 expression is suppressed in the thymus domain by the E10.5, [20]. suggesting that Tbx1 expression may be necessary for parathyroid formation but may antagonize rather than promote thymus development. Indeed, the ectopic expression of Tbx1 in TECs hampers their differentiation during thymus organogenesis [21].

In mice, by E10-11 a thymic rudiment becomes evident consisting of a single endodermal epithelial layer surrounded by neural crest-derived mesenchyme. Thereafter, hematopoietic progenitors start to colonize the thymus, triggering the formation of increasingly complex three-dimensional epithelial networks [22]. Developing thymocytes provide crucial signals for developing epithelial cells [23]. The presence of a thymus rudiment in FOXN1 KO mice embryos demonstrates that differentiation of the third pharyngeal pouch endoderm towards TECs occurs independently from FOXN1 expression. On the contrary, FOXN1

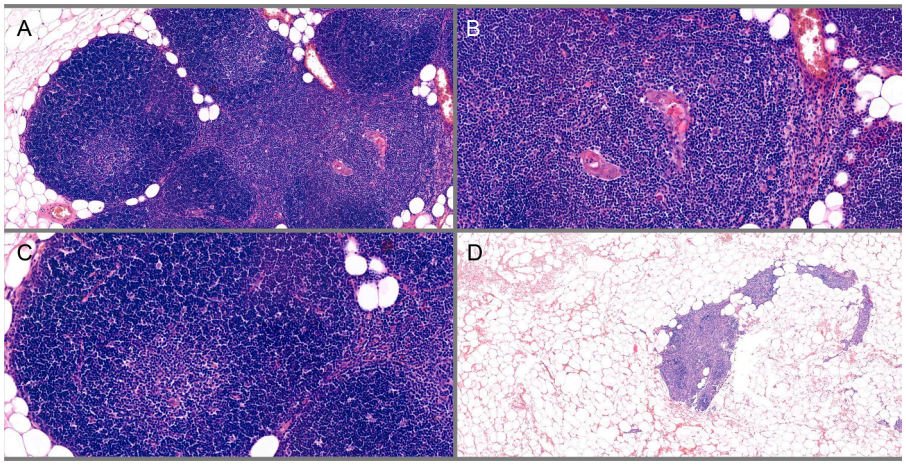

Fig. 1. Microscopical structure of the normal human thymus is shown by hematoxylin and eosin stained slides of formalin-fixed paraffined embedded samples showing: (A) the structural organization of the thymus in lobules with cortical and medullary regions; (B) the structure of the medulla with Hassall's corpuscles and epithelial cells mixed with thymocytes; (C) The structure of the cortex with densely pack thymocytes outnumbering thymic epithelial cells; (D) the features of thymic involution of the adulthood with fatty tissue replacement.

Table 1
Thymic epithelial cells with relative markers.

Population (Other names)	Organism	Gene expression	Reference
cTEC Dll4^{hi}	Mouse	Ly51, Ly75, CD40, CD83, MHCII, CCL25, CXCL12, SCF, IL7, Krt8, Prss16, Psmb11, Ctsl, Dll4^{hi}	[14,37,39,40].
cTEC Dll4^{lo}	Mouse	Ly51, Ly75, CD40, CD83, MHCII, CCL25, CXCL12, SCF, IL7, Krt8, Prss16, Psmb11, Ctsl, Dll4^{lo}	[14,37,39,40].
cTEC^{hi}	Human	HLA class II, PSMB11, PRSS16, CCL25, CD205	[3,14,75].
cTEC^{lo}	Human	HLA class II, PSMB11, PRSS16, CCL25, KI67+, CD205	[3,14,75].
Thymic Nurse Cells (TNC)	Mouse	Krt5, Krt8, Psmb11, MHCI, MHCII	[29,45,46].
Immature TEC-1	Human	FOXN1, PAX9, SIX1, ZBED2	[3,77].
Immature TEC-2	Human	FOXN1, PAX9, SIX1, IGFBP5, NNMT, MAOA, DPYS, FKBP5, GLUL, ZBED2	[3,77].
Pre-AIRE mTEC (jTEC, Intertypical TEC, mTECI)	Mouse	CD45-, EpCAM, UEA1, Pdpn, CCL21, CD104, Krt5, Krt10, MHCII^{lo}, CD80^{lo}	[13,30,32,33,61,63–65].
	Human	CD45-, EpCAM, KRT14+, GABRA5, LYPD1, KRT15 KRT8, KRT5, CCL21, LTBR, CD40	[3,74].
Transient amplifying TEC (TAC-TEC)	Mouse	CD45-, EpCAM, UEA1, Pdpn, CCL21 CD104, Krt5, Krt14, IGTB4, MHCII^{lo}, CD80^{lo}, Sca-1, Relb, Fezf2, Ascl1, Mki67, cyclins, E2f	[60,61].
Mature mTEC (mTECII)	Mouse	CD45-, EpCAM, UEA1, AIRE, Fezf2, MHCII^{hi}, CD80^{hi},CD40, CD74, CD52, Irf5, Nfκb2	[13,32,33,64,68–70].
	Human	CD45-, EpCAM, UEA-1, CD49fint/low, CD200, AIRE, FEZF2, SPIB, HLA class II, CLEC7A, MARCO, FXYD2, FXYD3, IL4I1, CHI3L1, CD70, TNFRSF9, RANK, OPG, CD40	[3,74–76].
Post-AIRE mTEC (mTECIII, Keratinocyte mTEC, Corneocyte-like mTEC)	Mouse	CD45-, EpCAM, UEA1, MHCII^{lo}, CD80^{lo}, Ly6d, Ivl, Krt10 and Spink5, Sbsn, Pigr, Cnfn, Flg	[13,29,32,33,60,61].
	Human	KRT1, KRT10, IVL, FXYD3, IL1RN, LYPD2, LTBR, CD40	[3,74].
Thymic Tuft Cell (mTEC IV, Tuft cell-like mTEC)	Mouse	Lrmp, Avil, Trpm5, Dclk1, Gng13, L1cam, Sox9,IL25, IL10, Pou2f3, MHC-II^{lo}, Chat	[32,33].
	Human	POU2F3, DCLK1, GNAT3, GNB3, PLCB2, OVOL3, TRPM5	[3,74].
Ciliated Cell	Mouse	Mcidas and Spag8, Dynlrb2 and Dnah12, FoxJ, Spag16, Wdr34, Bbs7, Tppp3, Fam183b	[33,61].
	Human	ATOH1, GFI1, LHX3, FOXJ1	[3].
Secretory/ Neuroendocrine cell (Neuro TEC)	Mouse	FoxA, Snap25, Stxbp5l, Klk1 and Krt7, Car8, CD177	[13,33].
	Human	NEUROD1, NEUROG1, CHGA, BEX1	[3,74].
Keratinocytes Grainyhead-like mTEC	Mouse	Cnfn, Flg and Grhl1/2/3, Ivl and Cnfn	[13,33,44,61].
Microfold mTEC	Mouse	Hnf4, Spi, Sox family, Tnfaip2, Tnfrsf11b, Ccl6, Ccl9, Ccl20, Gp2	[33,61].
Enterocyte/Hepatocyte mTEC	Mouse	Apoa4, Aldob, Reg3g and Saa1/Saa3	[33,61].
Skeletal muscle mTEC (Myoid Cell, myo TEC)	Mouse Human	Desmin, Myl1, Actc1, Myog MYOD1, MYOG, DES	[33,73]. [3,74].
Ionocyte mTEC	Mouse Human	Slc12a2 and Atp6v1b1 CFTR, FOXI1, ASCL3, CLCNKB, KRT8, TRPM2	[33,61]. [3].
Skin/Lung basal mTEC	Mouse	Aqp4, Aqp5, Muc5b, Slc12a2, Bpifa1	[33,61].
Pancreatic mTEC	Mouse	Ptf1a	[33,61].
Neural TEC (nTEC)	Mouse	Sod3, Dpt	[13].
Structural mTEC (sTEC)	Mouse	CD177, Car8, Col1a1, Dcn, Fbn1	[13].
Hassall's Corpuscles (HC, Thymic corpuscles)	Human	KRT10, Thymic stromal lymphopoietin (TSLP)	[72].
Myelin-positive cell	Human	SOX10, MPZ, MBP, S100A1	[3].

is a master regulator of TEC differentiation, [24]. including the formation of three-dimensional TEC networks and the efficient recruitment and differentiation of T cell progenitors [24]. Foxn1 is essential for initiating a transcriptional program that drives the early differentiation of TEC progenitors and guides the subsequent development of cortical and medullary cells through distinct stages of differentiation [17]. Alterations in FOXN1 lead to disruption of thymic architecture, as exemplified by the nude mice with thymic aplasia and severe combined immunodeficiency (SCID). In humans, germinal mutations of Foxn1 determine a similar phenotype with thymic aplasia [25]. All CD45-EPCAM+ TECs within the embryonic mouse thymus express Foxn1 [26]. TEC progenitors in the embryonic thymus are associated with the expression of Psmb11, encoding a thymus-specific component of the immunoproteasome [13].

Bipotent TECs become evident by E12 in mice [27]. The existence of bipotent TEC progenitors in the embryonic thymus is supported by experiments that demonstrate the ability of a single CD45-EPCAM+ cell, isolated from the mouse thymus at E12.5, to give rise to cTEC and mTEC after transplantation [28]. Fate-mapping approach confirmed that a single embryonic TEC can give rise to cTECs and mTECs [17]. Using the combination of scRNA-seq and a CRISPR–Cas9–based cellular barcoding the qualitative and quantitative changes in the thymic epithelium over time have been evaluated in mice. Two distinct progenitor populations have been identified: an early bipotent progenitor with a preferential differentiation towards cortical epithelium and a postnatal bipotent progenitor population preferentially oriented towards medullary epithelium [29]. At E16, cTEC signature and early progenitor cells are predominant whereas only in the newborn mice, the number of postnatal progenitors and mTECs begin to increase. At post-natal day (P) 28, postnatal TEC were more abundant than early progenitors, and cTECs were proportionally reduced being mTEC prevalent. TEC compartment exhibited signs of functional deterioration at 1 year of age, when mature cTECs and mTECs represent a small fraction of the thymic epithelia which is dominated by an unusually large number of cells simultaneously exhibited signatures of both progenitor types. The characteristics observed in aged mice suggest that the expanded progenitor-like cells may have lost their defining features. This indistinct phenotype appears to be associated with a reduced differentiation potential in these aged progenitors [29]. The continuous autocrine provision of Fgf7 leads to sustained expansion of TECs that express Fgfr2b without exhausting the progenitor pools, suggesting a strategy to modulate the extent of thymopoietic activity [29]. The preferential differentiation of post-natal progenitors towards mTEC was confirmed in different studies [17,28]. The ability of TEC progenitors expressing the β5t+ proteasome to expand and maintain the mTEC scaffold is progressively reduced in adolescent mice [30].

None of the adult mTEC subpopulations were present at E14.5 even if some AIRE-expressing cells can be observed as early as E13.5 [31,32]. Pre-AIRE and mature mTEC (AIRE+) become detectable by E18.5 and at P6, also tuft-like mTEC can be observed [32]. Newborn mice present a more modest enrichment for some mimetic cell subtypes, including muscle, enterocyte/hepatocyte, and ciliated mTECs [33].

During the first year of mouse life, TEC heterogeneity changes with 9 different TEC subtypes identified [13]. The cTEC compartment is composed of at least two main subtypes, designated perinatal and mature cTEC [13]. Perinatal cTEC make up a substantial proportion, accounting for approximately 40 % of all TEC in the first week after birth. However, their frequency diminishes rapidly thereafter, with only a minor fraction of these cells being observed in adult animals. Conversely, mature cTEC and jTEC increase in frequency and represent most of the cortical epithelia at 4 weeks, reaching approximately 30 % and 60 % of all TEC, respectively, by 1 year [13]. With aging, mature mTEC express genes involved in inflammatory signaling, apoptosis, and KRAS signaling, while reducing the expression of genes involved in cholesterol homeostasis and oxidative phosphorylation. jTEC displayed an opposite pattern as they aged, showing reduced cytokine signaling pathways and a stronger inflammatory signature characteristic of senescent tissues [34]. With age, mouse TEC progenitors become increasingly blocked in a stage resembling that of jTECs, expressing both cortical and medullary markers simultaneously [13].

3. Murine cTECs

Epithelial cells present in the cortex of the adult thymus are subclassified into cells that promote the differentiation of DN thymocytes, cells involved in positive selection, and TNCs [3,35].

Murine TECs are EPCAM + CD45- and the cortical population is defined by the expression of Ly51+ and UEA-1 markers on the cell surface and the expression of KRT8+ KRT5- and KRT14- [5,32].

Pro-T lymphocytes leave the bone marrow and enter the thymus from the cortical medullary junction and become DN1 thymocytes. Cortical TECs produce Cxcl12 and Ccl25 cytokines that promote the homing of pro-T lymphocytes into the thymus [36]. Thereafter, DN2 thymocytes migrate into the thymic cortex and activate the pattern of differentiation that leads to the rearrangement of variable portions of TCR chains. The interaction of NOTCH expressed on the cell membrane of pre-lymphocytes with Delta-like ligand 4 (Dll4) expressed by cTEC guides the differentiation of thymocytes [37].

In mice, mature cTECs can express different levels of Dll4 and therefore it has been suggested the division of cortical epithelial cells into $Dll4^{hi}$ (high expression) and $Dll4^{lo}$ (low expression) [37]. $Dll4^{hi}$ cells interact with DN2 thymocytes and promote TCR rearrangement through the DN3 and DN4 stages. On the contrary $Dll4^{lo}$ cells can interact with DP thymocytes and are involved in the process of positive selection [14]. The repertoire of TCRs generated through VDJ recombination undergoes functional screening through the binding with MHC-peptide complexes expressed on the surface of cTECs. For this purpose, cTECs express a unique proteasome known as the thymoproteasome and lysosomal proteases that facilitate an alternative cleavage and presentation of self-antigens. The components of the antigen-processing and presentation machinery expressed exclusively by cTEC include the thymoproteasome subunit β5t (encoded by Psmb11), cathepsin L1 (Ctsl), and thymus-specific serine protease encoded by Prss16 [38,39]. A relatively weak interaction between TCR and MHC self-peptide complex is necessary for the survival of DP thymocytes, which thereafter undergo clonal expansion and differentiate into either CD4 or CD8 positive cells. Conversely, thymocytes that fail the positive selection undergo apoptosis [14]. Moreover, cTECs express IL-7 and stem cell factor (Scf), which support the proliferation and survival of thymocytes [40]. Cortical TECs in the postnatal thymus express high levels of CD83, [41]. which regulates the turnover of surface MHC class II molecules, [14]. Ly51(known as CD249, encoded by *Enpep*) and Ly75 (also known as CD205).

In mice, most E14.5 TECs were relatively homogenous and expressed many cTEC-specific genes, however their general transcriptional signature was distinct from adult cTECs. A progressive downregulation of cell cycle genes and upregulation of the MHC-II pathway in adult cTECs was observed compared to embryonic cTECs [32]. Although cTECs in the postnatal thymus are EPCAM+, Ly75+, $MHC\text{-}II^{hi,}$ and $CD40^{hi}$, during embryogenesis, the expression levels of MHC-II and CD40 are lower [42]. ACKR4 (also known as CCRL1) is highly expressed during embryogenesis and becomes heterogeneous in the postnatal thymus. ACKR4 is a non-signaling decoy receptor for chemokines CC-chemokine ligand 19 (CCL19), CCL21 and CCL25, expressed in EPCAM+, Ly75+ cTECs [43]. Perinatal cTEC express Foxn1 and are constituted by multiple subpopulations expressing HVEM, Ly51, MHCII, CD83, and CD40 [44]. Three clusters of cTEC are described in the first week after born which reduces to a unique more homogeneous cluster by the fourth week [44]. Perinatal cTEC express Syngr1 and Gper1 [13].

TNCs are epithelial cells of the thymic cortex able to engulf several thymocytes, up to 200, within specialized vesicles. TNCs constitute approximately 10 % of β5t+ cTECs [45,46]. and can be found in various thymic microenvironments, spanning from the subcapsular region to the corticomedullary junction [35]. Their function has been poorly understood since their discovery in 1980 in a murine model [46]. TNCs express KRT5 and KRT8 supporting their epithelial nature. Despite their phagocytic capabilities, TNCs do not resemble macrophages or dendritic cells that usually perform such functions. Consequently, TNCs are epithelial cells capable of performing processes typically carried out by cells of the immune system. TNCs express both class I and class II MHC complexes on their cell membrane. The expression of class II MHC is intriguing since usually limited to professional antigen-presenting cells of the immune system. Within the engulfed vesicles, thymocytes can maturate into DP T cells [45]. DP thymocytes enclosed within thymic nurse cells undergo secondary TCR α-chain rearrangement. TNCs are less abundant in transgenic mice with impaired TCR rearrangement (Rag2deficent) or with a block of thymocyte differentiation in the DN1 stage (CD3ε Tgε26). This suggests that TNCs provide a microenvironment for optimal TCR repertoire selection by nurturing DP thymocytes to express the secondary rearranged TCR, thereby enabling a second chance of positive selection [45]. cTEC that differentiate in TNCs have a pronounced expression of CD3e, Prr16, CD205, Cxcl12, Tgfb, Tssp, and Vcam-1 [29,45].

4. Murine mTECs

mTECs are the effectors of negative selection: they perform PGE, ectopically expressing thousands of TRAs in a mosaic fashion. The immature auto-reactive thymocytes that recognize TRAs are eliminated by apoptosis or converted into regulatory Tregs, thereby preventing autoimmunity. Histologically, several types of mTEC can be recognized in the thymic medulla by light microscopy. In addition to canonical mTEC, other histological types have been described, including cornified, skin-like Hassall's corpuscles, ciliated cells, striated myoid cells, and electron-dense neurosecretory cells (Fig. 2) [33].

The presence of multiple types of epithelial cells in the thymic medulla has been confirmed by the analysis of surface markers by flow cytometry and more recently scRNA-seq revealed an even higher heterogeneity in the mTEC subpopulations. mTEC are defined as CD45-EPCAM + Ly51-UEA1+ cells in the mouse thymic medulla. mTECs originate from a bipotent progenitor that also gives rise to cTECs [17,28]. Beside embryonal progenitors, the presence of a common progenitor of cortical and medullary TECs after birth has been demonstrated [17]. mTECs with low expression of $MHCII^{lo}$ and $CD80^{lo}$ named pre-AIRE include the progenitor population.

A reciprocal crosstalk between TECs and thymocytes has been demonstrated. While mTECs orchestrate the negative selection of autoreactive thymocytes, autoreactive CD4+ SP cells are essential for

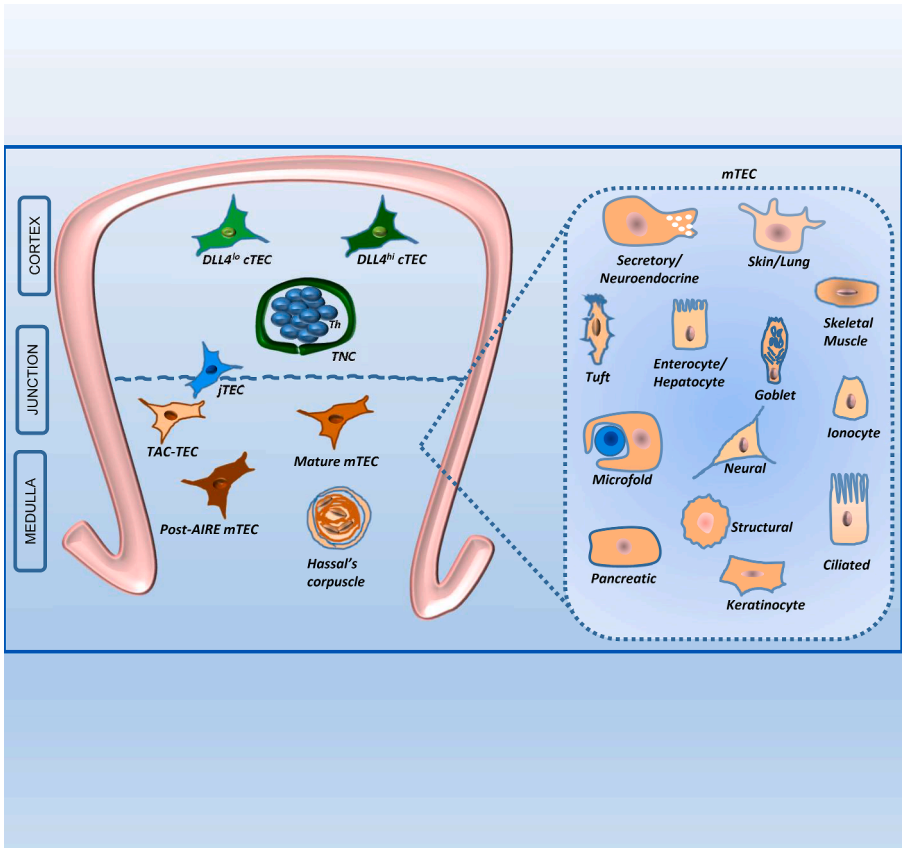

Fig. 2. Representation of the heterogeneity of thymic epithelial cells (TEC) in a thymic lobule. The cortex and the medulla are connected by the cortico-medullary junction (Junction). In each region resident epithelial cells have been depicted including medullary thymic epithelial cells (mTECs), cortical thymic epithelial cells (cTECs), and Junctional thymic epithelial cells (jTECs). A specific box for the numerous mimetic mTECs has been included. DLL4: Delta-like ligand 4; Thymic Nurse cell (TNC); Thymocyte (Th); Transient amplifying TEC (TAC-TEC); Autoimmune regulator (AIRE).

the differentiation of mTECs and the proper maturation of the thymic medulla [47–50].). The thymus of RAG1 and RAG2 knock-out mice has a normal cortex but a disorganized medulla with only a few scattered mTECs. RAG1 and RAG2 are necessary for TCR recombination, and T cells are absent in knock-out mice, resulting in SCID. However, after the transplantation of thymocyte precursors from bone marrow, there is complete development of the thymic cortex and medulla, demonstrating the importance of thymic crosstalk interactions [48]. In mice, CCR7 expressed by SP thymocytes, along with its two ligands, CCL19 and CCL21, expressed by mTECs, is responsible for the migration of SP thymocytes from the cortex to the medulla [51]. Mice knocked out for CCR7 or its ligands exhibit an arrest in thymocyte migration in the cortex and display abnormal medulla formation [52,53]. A defect in CD4+ SP thymocytes, rather than CD8+ SP thymocytes, affects the differentiation of mTECs, including the CD80hiAire− and CD80hiAire+ subsets [47]. CD4+ thymocytes control the differentiation of Aire+ $mTEC^{hi}$ cells by activating RANK and CD40-induced NF-κB signaling pathways [50]. In the embryonic mouse thymus, around the 16th day of gestation, lymphoid tissue inducer cells (CD3−CD4 + IL-7Rα+) regulate the development of the first Aire+ mTECs. These lymphoid tissue inducer cells express RANKL, which controls the maturation of Aire+ mTECs [54–56]. The absence of RANK or RANKL expression in the embryonic thymus results in a dramatic reduction of Aire+ mTECs and TRA expression [55,56]. On the contrary, RANK or RANKL knockout in the postnatal thymus only moderately decreases Aire+ mTECs. In contrast, CD40 and CD40L knockout results in a dramatic defect in mTEC subsets in postnatal mice [56,57]. Self-reactive CD4+ thymocytes control the composition of the $mTEC^{lo}$ compartment, which includes precursors of Aire+ $mTEC^{hi}$ cells, post-Aire cells, and tuft-like mTECs [50]. CD4+ thymocytes regulate maturational programs in mTEClo through MHCII/TCR interactions [50]. These antigen-specific interactions between mTECs and CD4+ thymocytes also necessitate engagement of the CD28–CD80/86 and CD40–CD40L costimulatory axes [58].

RANKL and CD40L expressed by CD4 SP thymocytes bind to the respective receptors on the surface of epithelial cells, activate the NF-KB pathway, and promote their differentiation into mature mTEC characterized by the expression of AIRE and $MHCII^{hi}$, $CD80^{hi}$ markers [57,59]. These cells express TRAs and participate in the negative selection. Thereafter, the mature mTECs suspend the expression of AIRE and become post-AIRE mTEC characterized by the expression of $MHCII^{lo}$ and $CD80^{hi}$ AIRE-. These cells can express corneocyte-like markers including Ly6d, Ivl, Krt10, and Spink5: markers enriched in the Hassall corpuscle. Moreover, mTEC can orient their differentiation in the well-described thymic tuft-like cells or other post-AIRE mimetic cells: cells expressing a pattern of genes that resemble that of cells present in other organs. Mimetic cells can include neuroendocrine-like, secretory cell types, keratinocytes grainyhead-like, microfold, enterocyte/hepatocyte, skeletal muscle, ionocyte, skin/lung basal, goblet, pancreatic, and fibroblast-like mTEC [11,13,31–33,44,60–62].

The expression of podoplanin (Pdpn+) and Ccl21+ can distinguish pre-AIRE mTEC from post-AIRE mTEC being both $MHCII^{lo}$, $CD80^{lo}$, and AIRE- cells. Some authors subdivide pre-AIRE TEC into 2 clusters of cells: one able to maturate in transient amplifying TEC and thereafter in mature mTEC and the other with less proliferative potential that is supposed to remain resident in the thymus [61]. The secretion of CCL21 creates a gradient able to attract SP thymocytes maturated in the cortex that express the CCR7 [63]. The pre-AIRE mTEC have been named junctional TEC (jTEC) or intertypical TEC because they expressed medullary and cortical markers and their ability to differentiate into

mTEC and cTEC [63]. jTECs have a progenitor potential and are localized at the cortico-medullary junction [30]. In adult mice, jTECs are MHCII^{lo}, CD80^{lo}, AIRE- and express Pdpn, CD104, Krt10, and the transcription factors Sox4, Ascl1, Irf7, and Irf9 [33,61,64]. The cluster of cells highly expressing Ccl21a, Krt5, and Krt14 have been defined as MHC-II^{lo}, ITGB4+, L1CAM− [65]. or CD45-, EpCAM+, Ly51-, CD80^{lo}, MHCII^{lo}, DSG3- [13]. The lymphotoxin β receptor regulates the development of CCL21 expressing mTEC [65]. Ccl21a-expressing cells give rise to Aire-expressing mTECs [63]. However, the disruption of the Ccl21a-expressing population has been demonstrated to have minimal impact on the Aire-expressing compartment, suggesting that there may not be a precursor–product relationship [65].

Transient amplifying mTEC are rapidly proliferating cells as demonstrated by the expression of Mki67, cyclins, E2f, and other genes implicated in the G2/M phases of the cell cycle [61]. Transient amplifying TECs start to express Aire^{lo} but at an inferior level than mature mTEC. Moreover, transient amplifying TECs express Sca-1, Relb, Fezf2, and Ascl1 and recently have been found to have self-renewal potential and the ability to differentiate in cTEC and mTEC [66].

Mature mTEC represent 60 % of mTEC, express Aire and Fezf2 performing PGE, and phenotypically are defined by CD45-, EpCAM+, Ly51-, MHCII^{hi} and CD80^{hi}. The mature population of Aire+ mTECs exhibits significant inherent diversity, crucial for their role in eliminating self-reactive thymocytes through negative selection and promoting the selection of self-reactive clones. The expression of TRAs by mTECs, a process regulated by AIRE and FEZF2, contributes to this diversity. Despite each mTEC expressing only a subset of TRAs, mosaic coverage is required to optimize selection efficiency [67]. PGE in the medulla is a biologically indeterminate yet ordered process whose order is provided by repeated co-expression of gene subsets. AIRE regulates the expression of TRAs, but its functions extend beyond, indicating a crucial involvement in the development and differentiation of mTECs [68]. Two debated paradigms regarding how mTECs present TRAs to T-cells in the thymus have been proposed. The first suggests a "stochastic" pattern where individual mTECs randomly express various TRAs. The second paradigm supports a "coordinated" pattern of TRAs expression by specific mTECs in a synchronized manner based on their biological properties [68]. These co-expression of TRA genes is not systematically collocated in the linear genome and are not linked by tissue specificity, or by biological or disease processes, even if an ordinate program of expression in different mTEC is suggested from data of scRNA-Seq [61]. Beside Aire and Fezf2, mature mTEC express Cd40, H2-Aa or Cd74, Cd52, Irf5 and Nfκb2 [13,33,64]. The interaction with RANKL and CD40L expressed on the surface of CD4 SP thymocytes activates NF-KB and prompts the differentiation of precursor cells in mature mTEC [57,59,69]. Indeed, the differentiation of mature mTECs is selectively blocked after treatment with an anti-RANK-ligand (RANKL) antibody [70]. In the adult thymus, mature mTECs have an estimated half-life of 12–14 days. [71].

Post-AIRE cells suspend the expression of Aire but still express TRA even if they are CD45-EPCAM+, MHCII^{lo}, and CD80^{lo}. Post Aire cells can be defined as Itgb4−L1cam−Ly6d+ [32]. or CD45-, Epcam+, Ly51-, CD80^{lo}, MHCII^{lo}, and Dsg3+ [13]. Post Aire cells express corneocyte-like markers including Pigr, Ly6d, Spink5, Ivl, Krt10, and Sbsn. Spink5 has previously been found in Hassall's corpuscles [72]. and appears to be a more informative indicator of terminally differentiated mTEC in our dataset than the classically used Ivl and Krt10 transcripts.

Aire knockout (KO) mice experiments confirmed that AIRE deficiency significantly reduced the expression of TRA genes within mature and post-AIRE mTEC. mTECs exhibiting high levels of MHCII and CD80 were elevated in Aire-deficient mice. AIRE deficiency also led to a decline in pre-AIRE and post-AIRE mTEC populations without relevant impact on the tuft cell-like mTEC [33].

Tuft cell-like mTECs express a pattern of genes typical of the Tuft cell found in other tissue mainly the gut including Lrmp, Avil, Trpm5, Dclk1, Gng13, L1cam, and Sox9 [32,33]. Tuft cell-like mTECs are defined as MHC-II^{lo}, L1CAM+. Pou2f3 is a master regulator of tuft cell differentiation and these mTEC express such transcription factor. Mice KO for Pou2f3 have a selective deficiency of tuft cell-like mTECs in the thymus whereas other types of mTEC are not affected. Tuft cell-like mTECs express IL25 and IL10. Pou2f3 KO mice have an increased number of innate lymphoid cells, cells without IL25 receptor (IL25R), in the thymus whereas there was not a significant impact on other thymic CD45+IL-25R+ cells or the main T cell subsets [32].

Additional subsets of mTEC with a post-AIRE phenotype Aire^{lo}, Pdpn^{-}, CD104- and accessibility at post-Aire markers like Pigr and Spink5 have been described with a non-identical definition among different analyses. Michelson D. A. et al named them "mimetic cells" because partially recapitulate gene expression typical of specific cells of other organs.

Ciliated cell-like mTECs express Mcidas and Spag8, essential regulators of ciliogenesis, Dynlrb2, Dnah12, and FoxJ. In Foxj1YFP mice, YFP expression localized to clusters of polarized, acetylated-tubulin-positive, ciliated cells surrounding cysts, consistent with old reports of ciliated cysts in the thymus [33].

Secretory/neuroendocrine cells are defined by the expression of FoxA, Snap25, and Stxbp5l and exhibit enriched accessibility to secretory-cell-specific genes like Klk1 and Krt7, by Assay of Transposase Accessible Chromatin sequencing (ATACseq), encoding characteristic markers of glandular epithelium [13,33,61]. Keratinocytes Grainyhead-like mTECs express Cnfn, Flg, and Grhl1/2/3 genes important for the control of skin development. Keratinocytes Grainyhead-like mTEC showed enriched accessibility at keratinocyte-specific genes Ivl and Cnfn, by ATACseq, genes encoding key components of the cornified cell envelope in the skin [33,44,61].

Microfold mTECs are scattered diffusely throughout the medullary regions and, according to ATACseq, are enriched for Hnf4, Spi, and Sox family motifs. Therefore, these cells are reminiscent of gut microfold (M) cells, professional antigen-transcytosis cells that differentiate in Peyer's patches from Hnf4-expressing intestinal epithelial cells in a SpiB- and Sox8-dependent fashion. Microfold mTECs express Tnfaip2, Tnfrsf11b, Ccl6, Ccl9, and Ccl20. Enterocyte/hepatocyte mTEC are enriched for accessibility by ATACseq to Hnf4 and to enterocyte- and hepatocyte-specific genes like Apoa4 and Aldob, encoding lipid- and sugar-processing proteins produced by the gut and liver. Enterocyte/hepatocyte mTEC express Reg3g and Saa1/Saa3 [33,61].

Skeletal muscle mTECs are desmin-positive cells scattered through the medulla and express Myl1 and Actc1. These cells could represent the previously histologically described "myoidzellen" [73]. Finally, additional clusters of mTEC include Ionocyte mTEC expressing Slc12a2 and Atp6v1b1, Skin/lung basal mTECs, Goblet mTECs, pancreatic mTEC expressing Ptf1a, neural TEC expressing Sod3, Dpt and structural mTEC expressing Col1a1, Dcn, Fbn1 [13,33,61].

5. Human TEC

In humans, the expression of EPCAM is higher in mTEC and lower in cTEC (Table 2) [74]. The co-staining of EPCAM with KRT5 and KRT8, specific markers of TEC, reveals some cytokeratin-positive cells with low

Table 2
Expression of EPCAM, FOXN1, and specific markers in human thymic epithelial cell subtypes.

TEC subtype	EPCAM Expression	FOXN1 Expression	Gene expression marker
cTEC	EPCAM+	FOXN1++	PSMB11
mTEC(I)	EPCAM+	FOXN1+/-	KRT14
mTEC(II)	EPCAM++	FOXN1+/-	AIRE+
mTEC(III)	EPCAM+	FOXN1+/-	KRT1
mTEC(IV)	EPCAM+++	FOXN1+/-	FOXI1
TEC(myo)	EPCAM+	FOXN1+/-	MYOD1
TEC(neuro)	EPCAM++	FOXN1+/-	NEUROD1, CHGA

expression of EPCAM [75]. Therefore, the expression of EPCAM seems insufficient to identify all human TEC. It has been proposed for the identification of TEC the staining with anti-PDPN in conjunction with anti-EPCAM antibodies [75]. Fibroblast and lymphatic endothelial cells also express PDPN. For the identification of cTEC and mTEC, it has been reported that UEA-1 label human mTEC [76]. and human cTECs can be identified using anti-CD205 or CDR2 antibodies [14].

However, in the human thymus, the staining with anti-UEA-1 and anti-CD205 left a substantial fraction of TECs unstained, and since UEA-1 was exclusively found in the medulla it failed to label all mTECs. It has been proposed that antibodies reactive to CD200 (on mTEC/EpCAM^{hi}) and CD49f (on cTEC/EpCAM^{lo}) respectively proved to be most effective due to high expression levels and low inter-individual variability [75]. Indeed, fibroblast, endothelial, and some B cells also express CD200, and reactivity to CD49f was detected in intrathymic vascular structures. Therefore, it has been proposed that PDPN$^{high/int}$EpCAM$^{high/int/low}$ identify TECs, and thereafter CD49f+CD200- define cTECs and CD49fint/lowCD200+ mTECs. scRNA-Seq demonstrated a similar organization between human and murine TEC [3,74]. Human TEC were enriched using CD3-CD45-EPCAM+ expression. Conserved TEC populations were identified across species, including PSMB11-positive cTECs, KRT14+positive pre-AIRE mTECs (mTEC-I according to Bronstein), AIRE-expressing mature mTECs (mTEC-II), and KRT+ expressing post-AIRE mTECs (mTEC-III) [74]. Tuft-like mTEC (mTEC-IV according to Bornstein) were a very rare population in humans. Interestingly, cTECs were more abundant during prenatal weeks 7 and 8, and an intermediate population of mTEC expressing DLK2 was evident in late fetal and pediatric human thymus [74]. The expression of DCLK1 or POU2F3 that well defines these cells in mice seems to be less specific in humans. Some “mimetic cells” have been described in human thymi including myoid TEC expressing MYOD1+ and MYOG+, neuroendocrine TEC expressing NEUROD1+, NEUROG1+, and CHGA+ [74].

Bautista JL et al. further increase the resolution of TEC clustering in humans by identifying two subsets of PSMB11 positive cTECs according to the expression of functional genes: HLA class II, PSMB11, PRSS16, CCL25: cTEC^{lo} and cTEC^{hi}. The cTEC^{lo} present increased proliferative KI67+ cells [3].

Pre-AIRE mTEC were defined by the expression of CLDN4, lower levels of HLA class II, and included a subpopulation that expressed high levels of the chemokine CCL21 like what was observed in murine jTEC [3,64]. Genes enriched in Pre AIRE mTEC include GABRA5 and LYPD1. A cluster of cells, named immature TEC, was identified by the expression of the canonical TEC genes FOXN1, PAX9, and SIX1 in the absence of functional genes of cTECs or mTECs. These immature cells possibly represent progenitors that are not committed to a specific lineage or cells that have lost their differentiated phenotype over time [3].

With an even higher resolution, two clusters of immature TEC (TEC-1 and TEC-2) were identified with distinct markers. The expression of IGFBP5, NNMT, MAOA, DPYS, FKBP5, and GLUL was enriched in immature TEC-2 and prominent in adult cells compared to fetal and postnatal tissues. The ZBED2 was identified as a gene highly expressed in both immature TECs subsets, interestingly it is a zinc-finger transcription factor without a murine counterpart that has recently been linked to the maintenance of the basal state in human keratinocytes [77]. The expression of KRT15 was confirmed in mTEC^{lo} progenitors and located using immunofluorescence into the medulla KRT15^{hi} likely in CCL21+ mTEC^{lo} and with a lower expression into the corticomedullary junction KRT15^{lo} in KRT8+/KRT5+ cells.

Mature mTECs perform PGE and express, AIRE, FEZF2, SPIB, and high levels of HLA class II. Genes enriched in mature AIRE mTEC include CLEC7A, MARCO, FXYD2, FXYD3, IL4I1, CHI3L1, CD70, and TNFRSF9. Post-AIRE defined also corneocyte-like mTECs markers are KRT1 and IVL and are encircled for FXYD3, IL1RN, and LYPD2 expression. In adult thymus, there are a limited number of cTECs or mTEC^{hi}, suggesting an accumulation of immature TECs to the detriment of functional TECs in older tissue. Tuft-like mTECs are positive for GNB3, TRPM5, GNAT3, PLCB2, OVOL3, and POU2F3, similar to what is observed in murine thymus. Ionocyte mTECs express FOXI1, ASCL3, CFTR, CLCNKB. Ionocytes have been described in lung tissue but have not been previously described in the thymus, therefore, they have been confirmed using immunofluorescence with KRT8+/CFTR+ and TRPM2+/ CFTR+ staining into the human thymic medulla. Ionocytes were isolated cells in the medulla or were part of Hassall’s corpuscles [3]. The presence of neuroendocrine mTEC was defined by the expression of BEX1 and NEUROD1, of muscle-like myoid by the expression of MYOD1 and DES, and of myelin+ epithelial cells by the expression of SOX10 and MPZ. Neuroendocrine and a subset of myoid mTEC were also detected near the Hassall’s corpuscles [3]. Ciliated TECs are positive for ATOH1, GFI1, LHX3, and FOXJ1, whereas myelin+ cells for SOX10, MPZ, MBP, and S100A1 closely resemble Schwann cells [3].

In the murine thymus, RANK, CD40, and osteoprotegerin activate TNF receptor superfamily signals to maintain AIRE+ mTEC^{hi} cells [55]. Lymphotoxin B is necessary for the maturation of, CCL21 + mTEC^{lo} [65]. In humans, similarly, the Lymphotoxin B receptor LTBR can be detected in CCL21+ mTEC^{lo} and corneocyte-like mTECs while mTECs^{hi} express RANK and OPG, two receptors for RANKL and osteoprotegerin, respectively. Both mTEC^{lo} and mTEC^{hi} express CD40. There is a significant crosstalk between thymic fibroblast and epithelial cells. Fibroblast express many ligands (WNT5A, RSPO3, SFRP2, IGF1, and FGF10) for receptor expressed on epithelial cells (ROR1, ROR2, RYK, IGF1R, and FGFR2) [3]. The prediction of cellular interaction suggested that lymphotoxin signaling comes from diverse immune cells and is received by most of the stromal cells [74]. On the contrary, RANKL-RANK was confined between innate lymphoid cells group 3 and mature mTEC [74]. Fibroblast express FGF7, necessary for the growth of FGFR2 expressing TEC [74]. NOTCH1 is expressed in early thymic progenitors, and diverse Notch ligands are expressed by different cell types including cTECs and endothelial cells that express both JAG2 and DLL4, whereas JAG1 is broadly expressed by other TECs [78].

BMP4, FGF7, and the WNT inhibitor FRZB are expressed more frequently in postnatal and adult mesenchymal cells compared to fetal mesenchyme, suggesting that TEC differentiation and proliferation are differentially regulated by mesenchymal factors over time [3]. Mesothelial cells expressed BMP4 and the WNT signaling modulators RSPO1, RSPO3, SFRP2, and SFRP5 while pericytes expressed FRZB, WNT6, BMP5, FGF7, and INHBA (Activin A). Follistatin (FST), an activin antagonist, promotes TEC progenitor maintenance and inhibits differentiation [79]. FST is found mostly in adult mesenchymal cells and a subset of epithelial cells suggesting that human mesenchyme, endothelial cells, and pericytes provide factors critical for TEC development or through expression of chemokines and adhesion molecules that regulate migration of hematopoietic progenitors. Moreover, the extracellular matrix seems to contribute to the formation and maintenance of specialized compartments of the thymus fostering cellular processes including adhesion, migration, lineage commitment, and cell-cell interactions [80].

6. Single-cell RNA sequencing in thymoma

Thymic epithelial tumors are rare but heterogeneous neoplasms, including thymomas and thymic carcinomas. The formers are further classified into A, AB, B1, B2, B3, micronodular, and metaplastic histotypes. Thymic carcinomas predominantly exhibit squamous differentiation. Rare histotypes of carcinomas include basaloid carcinoma, lymphoepithelial carcinoma, clear cell carcinoma, low-grade papillary carcinoma, adenocarcinoma, mucoepidermoid carcinoma, adenoid cystic carcinoma-like, enteric-type adenocarcinoma, adenosquamous, and sarcomatoid carcinoma, according to the fifth edition of the WHO classification of thoracic tumors. Neuroendocrine tumors are classified as distinct entities, encompassing typical and atypical carcinoids, large-cell neuroendocrine carcinoma, and small-cell neuroendocrine carcinoma of the thymus [81]. The heterogeneity observed in thymic

epithelial tumors is not surprising, given the numerous subpopulations of TECs described in both humans and mice. The complexity of thymomas has been evaluated using SC-RNA-Seq in cells derived from patients with myasthenia gravis. These cells originate from a pool of four patients with two AB, a B1, and a B2 thymoma [82]. Stromal cells encompass a variety of cell types, including endothelial cells (positive for PECAM1/CD31, VWF), normal fibroblasts (FN1, EGFL6), tumor-associated fibroblasts (TAFs; PDGFRA, ADH1B), and TECs (TECs; KRT19, S100A14). Epithelial cells have been further subclassified into cTEC and mTEC, distinguished by the expression of CCL25+ PSMB11 for cTEC and CCL19+ KRT7 for mTEC, respectively. Medullary TEC were divided into cluster I, expressing KRT15 and IFI27, cluster II expressing CLDN4 and KRT7, and a cluster peculiar to thymoma named neuromuscular TEC (nmTEC), positive for GABRA5+ and KRT6+. nmTEC expressed brain-specific genes, such as GABRA5, MAP2, NEFL, NEFM, SOX15, TF [82]. In comparison to normal thymus, nmTECs are partially correlated with immature TECs and not with myoid cells or neuroendocrine TECs. Indeed, thymomas typically onset within adulthood, during which normal TECs predominantly exhibit impaired differentiation expressing cortical and medullary markers simultaneously [13]. According to immunohistochemistry, the expression of neuromuscular molecules is not specific to thymomas occurring in patients with myasthenia but can also be observed in other thymic epithelial tumors. Using scRNA-Seq clusters, bulk RNA sequencing data [83]. has been deconvoluted, showing that cTECs accumulate in A thymomas; mTEC(I) in A and B3 thymomas and thymic carcinomas; mTEC(II) in type A; and immature T cells in type B1 thymomas [82]. nmTECs and tumor-associated fibroblasts exhibited a distinct preference for expressing CXCL12, while thymic B cells and various T helper, including follicular T helper and T_{reg}, manifested the expression of its receptor, CXCR4. The significance of the CXCR4-CXCL12 axis has been demonstrated in T-cell homing within synovial tissues in rheumatoid arthritis, neurogenesis, and the maintenance of hematopoietic stem cells, suggesting that the interaction might play a crucial role in nmTEC-mediated T-cell regulation [82]. Furthermore, it has been predicted a potential interaction between nmTECs and vascular endothelial cells through VEGFA and VEGFE, as well as with tumor-associated fibroblasts through PDGFA-PDGFRA. Immunostaining on thymoma sections from myasthenic patients described a proximity of GABRA5+ nmTECs with CD31+ vascular endothelial cells suggesting that nmTECs could contribute to angiogenesis through interactions with vascular endothelial cells [82].

The L424H mutation of GTF2I is the most common in thymomas, especially in A and AB types [84,85]. Transgenic mice expressing the mutated GTF2I in TECs under the FOXN1 promoter have been generated by two independent groups [86,87]. Both transgenic mice developed thymomas, demonstrating that mutated GTF2I functions as an oncogene. The phenotype of mouse TECs expressing the mutant Gtf2i at 4-6 weeks after birth resembles an A-type-like thymoma, although some features of AB and B thymomas were also observed [86]. In the other transgenic model, murine thymoma features seem compatible with B1 thymomas with focal areas of B2 differentiation [87]. Gtf2i mutation causes an incomplete block of TEC differentiation, leading to an accumulation of immature Ly51–UEA-1– TECs [86]. Immunohistochemical analysis revealed numerous KRT5+KRT18+ DP epithelial cells characteristic of undifferentiated thymic epithelium [86]. Flow cytometric studies revealed a paucity of Ly51–UEA-1+ mTECs, which should predominate at this age in normal thymus, and a relative increase of Ly51 + UEA-1– cTECs [86]. Therefore, the Gtf2i mutation perturbs the differentiation of the epithelial compartment, most prominently affecting the emergence of mTECs, resulting in a microenvironment that is dominated by cTECs. The presence of a large fraction of undifferentiated TECs suggests an accumulation of progenitor cells [86,87].

While preliminary sc-RNA-Seq evaluations of thymomas indicate the proliferation of elements with impaired differentiation expressing progenitor markers of cortical and medullary origin, additional evaluations in humans are warranted to better characterize the tumor cells that make up different kinds of thymomas and thymic carcinomas.

7. Conclusions

Characterizing the subpopulations of TECs is useful for a comprehensive understanding of thymus function at the cellular level, encompassing crucial processes such as thymocyte maturation, positive and negative selection. Furthermore, elucidating the diverse subpopulations of TECs holds significant relevance in deciphering the biology of thymic epithelial tumors. Specifically, identifying the cell of origin for these neoplasms and comparing the characteristics of tumor cells with those of their normal counterparts, such as examining gene expression and methylation status, can provide invaluable insights into the underlying mechanisms of thymic epithelial tumorigenesis. Thus, continued research efforts aimed at unraveling the complexities of TEC subpopulations are essential for advancing our understanding of both thymus physiology and pathology.

CRediT authorship contribution statement

Eleonora Pardini: Writing – original draft, Investigation, Conceptualization. **Serena Barachini:** Writing – original draft, Investigation. **Greta Alì:** Writing – original draft, Data curation. **Gisella Sardo Infirri:** Supervision, Conceptualization. **Irene Sofia Burzi:** Conceptualization. **Marina Montali:** Supervision, Methodology, Conceptualization. **Iacopo Petrini:** Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

References

[1] L. Klein, B. Kyewski, P.M. Allen, K.A. Hogquist, Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see), Nat. Rev. Immunol. 14 (6) (2014) 377–391.
[2] P. Bhalla, D.M. Su, N.S.C. van Oers, Thymus functionality needs more than a few TECs, Front. Immunol. 13 (2022) 864777.
[3] J.L. Bautista, N.T. Cramer, C.N. Miller, J. Chavez, D.I. Berrios, L.E. Byrnes, J. Germino, V. Ntranos, J.B. Sneddon, T.D. Burt, J.M. Gardner, C.J. Ye, M. S. Anderson, A.V. Parent, Single-cell transcriptional profiling of human thymic stroma uncovers novel cellular heterogeneity in the thymic medulla, Nat. Commun. 12 (1) (2021) 1096.
[4] W. Savino, D.A. Mendes-Da-Cruz, S. Smaniotto, E. Silva-Monteiro, D.M. Villa-Verde, Molecular mechanisms governing thymocyte migration: combined role of chemokines and extracellular matrix, J. Leukoc. Biol. 75 (6) (2004) 951–961.
[5] E.N. Lee, J.K. Park, J.R. Lee, S.O. Oh, S.Y. Baek, B.S. Kim, S. Yoon, Characterization of the expression of cytokeratins 5, 8, and 14 in mouse thymic epithelial cells during thymus regeneration following acute thymic involution, Anat. Cell Biol. 44 (1) (2011) 14–24.
[6] K. Takada, I. Ohigashi, M. Kasai, H. Nakase, Y. Takahama, Development and function of cortical thymic epithelial cells, Curr. Top. Microbiol. Immunol. 373 (2014) 1–17.
[7] N.L. Alves, N.D. Huntington, H.R. Rodewald, J.P. Di Santo, Thymic epithelial cells: the multi-tasking framework of the T cell "cradle", Trends. Immunol. 30 (10) (2009) 468–474.
[8] L. Klein, M. Hinterberger, G. Wirnsberger, B. Kyewski, Antigen presentation in the thymus for positive selection and central tolerance induction, Nat. Rev. Immunol. 9 (12) (2009) 833–844.
[9] T.M. McCaughtry, T.A. Baldwin, M.S. Wilken, K.A. Hogquist, Clonal deletion of thymocytes can occur in the cortex with no involvement of the medulla, J. Exp. Med. 205 (11) (2008) 2575–2584.
[10] J. Derbinski, J. Gäbler, B. Brors, S. Tierling, S. Jonnakuty, M. Hergenhahn, L. Peltonen, J. Walter, B. Kyewski, Promiscuous gene expression in thymic epithelial cells is regulated at multiple levels, J. Exp. Med. 202 (1) (2005) 33–45.
[11] S.N. Sansom, N. Shikama-Dorn, S. Zhanybekova, G. Nusspaumer, I.C. Macaulay, M. E. Deadman, A. Heger, C.P. Ponting, G.A. Holländer, Population and single-cell genomics reveal the Aire dependency, relief from Polycomb silencing, and distribution of self-antigen expression in thymic epithelia, Genome Res. 24 (12) (2014) 1918–1931.
[12] H. Takaba, Y. Morishita, Y. Tomofuji, L. Danks, T. Nitta, N. Komatsu, T. Kodama, H. Takayanagi, Fezf2 orchestrates a thymic program of self-antigen expression for immune tolerance, Cell 163 (4) (2015) 975–987.

[13] J. Baran-Gale, M.D. Morgan, S. Maio, F. Dhalla, I. Calvo-Asensio, M.E. Deadman, A. E. Handel, A. Maynard, S. Chen, F. Green, R.V. Sit, N.F. Neff, S. Darmanis, W. Tan, A.P. May, J.C. Marioni, C.P. Ponting, G.A. Holländer, Ageing compromises mouse thymus function and remodels epithelial cell differentiation, Elife 9 (2020).

[14] N. Kadouri, S. Nevo, Y. Goldfarb, J. Abramson, Thymic epithelial cell heterogeneity: TEC by TEC, Nat. Rev. Immunol. 20 (4) (2020) 239–253.

[15] J. Lee, D.Y. Hyeon, D. Hwang, Single-cell multiomics: technologies and data analysis methods, Exp. Mol. Med. 52 (9) (2020) 1428–1442.

[16] L.M. Mustachio, J. Roszik, Single-cell sequencing: current applications in precision onco-genomics and cancer therapeutics, Cancers (Basel) 14 (3) (2022).

[17] C.C. Bleul, T. Corbeaux, A. Reuter, P. Fisch, J.S. Mönting, T. Boehm, Formation of a functional thymus initiated by a postnatal epithelial progenitor cell, Nature 441 (7096) (2006) 992–996.

[18] G. Giardino, C. Borzacchiello, M. De Luca, R. Romano, R. Prencipe, E. Cirillo, C. Pignata, T-cell immunodeficiencies with congenital alterations of thymic development: genes implicated and differential immunological and clinical features, Front. Immunol. 11 (2020) 1837.

[19] L.A. Jerome, V.E. Papaioannou, DiGeorge syndrome phenotype in mice mutant for the T-box gene, Tbx1, Nat. Genet. 27 (3) (2001) 286–291.

[20] F. Vitelli, M. Morishima, I. Taddei, E.A. Lindsay, A. Baldini, Tbx1 mutation causes multiple cardiovascular defects and disrupts neural crest and cranial nerve migratory pathways, Hum. Mol. Genet. 11 (8) (2002) 915–922.

[21] K.A. Reeh, K.T. Cardenas, V.E. Bain, Z. Liu, M. Laurent, N.R. Manley, E.R. Richie, Ectopic TBX1 suppresses thymic epithelial cell differentiation and proliferation during thymus organogenesis, Development 141 (15) (2014) 2950–2958.

[22] H.R. Rodewald, Thymus organogenesis, Annu. Rev. Immunol. 26 (2008) 355–388.

[23] V. Marrella, P.L. Poliani, L.D. Notarangelo, F. Grassi, A. Villa, Rag defects and thymic stroma: lessons from animal models, Front. Immunol. 5 (2014) 259.

[24] T. Corbeaux, I. Hess, J.B. Swann, B. Kanzler, A. Haas-Assenbaum, T. Boehm, Thymopoiesis in mice depends on a Foxn1-positive thymic epithelial cell lineage, Proc. Natl. Acad. Sci. U. S. A. 107 (38) (2010) 16613–16618.

[25] C. Pignata, A. Fusco, S. Amorosi, Human clinical phenotype associated with FOXN1 mutations, Adv. Exp. Med. Biol. 665 (2009) 195–206.

[26] I. Rode, V.C. Martins, G. Küblbeck, N. Maltry, C. Tessmer, H.R. Rodewald, Foxn1 protein expression in the developing, aging, and regenerating thymus, J. Immunol. 195 (12) (2015) 5678–5687.

[27] S. Ulyanchenko, K.E. O'Neill, T. Medley, A.M. Farley, H.J. Vaidya, A.M. Cook, N. F. Blair, C.C. Blackburn, Identification of a bipotent epithelial progenitor population in the adult thymus, Cell Rep. 14 (12) (2016) 2819–2832.

[28] S.W. Rossi, W.E. Jenkinson, G. Anderson, E.J. Jenkinson, Clonal analysis reveals a common progenitor for thymic cortical and medullary epithelium, Nature 441 (7096) (2006) 988–991.

[29] A. Nusser, J.B.S. Sagar, B. Krauth, D. Diekhoff, L. Calderon, C. Happe, D. Grün, T. Boehm, Developmental dynamics of two bipotent thymic epithelial progenitor types, Nature 606 (7912) (2022) 165–171.

[30] C.E. Mayer, S. Žuklys, S. Zhanybekova, I. Ohigashi, H.Y. Teh, S.N. Sansom, N. Shikama-Dorn, K. Hafen, I.C. Macaulay, M.E. Deadman, C.P. Ponting, Y. Takahama, G.A. Holländer, Dynamic spatio-temporal contribution of single β5t + cortical epithelial precursors to the thymus medulla, Eur. J. Immunol. 46 (4) (2016) 846–856.

[31] E.M. Kernfeld, R.M.J. Genga, K. Neherin, M.E. Magaletta, P. Xu, R. Maehr, A single-cell transcriptomic atlas of thymus organogenesis resolves cell types and developmental maturation, Immunity 48 (6) (2018), 1258-1270.e6.

[32] C. Bornstein, S. Nevo, A. Giladi, N. Kadouri, M. Pouzolles, F. Gerbe, E. David, A. Machado, A. Chuprin, B. Tóth, O. Goldberg, S. Itzkovitz, N. Taylor, P. Jay, V. S. Zimmermann, J. Abramson, I. Amit, Single-cell mapping of the thymic stroma identifies IL-25-producing tuft epithelial cells, Nature 559 (7715) (2018) 622–626.

[33] D.A. Michelson, K. Hase, T. Kaisho, C. Benoist, D. Mathis, Thymic epithelial cells co-opt lineage-defining transcription factors to eliminate autoreactive T cells, Cell 185 (14) (2022), 2542-2558.e18.

[34] C. Franceschi, M. Bonafè, S. Valensin, F. Olivieri, M. De Luca, E. Ottaviani, G. De Benedictis, Inflamm-aging. An evolutionary perspective on immunosenescence, Ann. N. Y. Acad. Sci. 908 (2000) 244–254.

[35] J.C. Guyden, M. Martinez, R.V. Chilukuri, V. Reid, F. Kelly, M.O. Samms, Thymic nurse cells participate in heterotypic internalization and repertoire selection of immature thymocytes; their removal from the thymus of autoimmune animals may be important to disease etiology, Curr. Mol. Med. 15 (9) (2015) 828–835.

[36] W.E. Jenkinson, S.W. Rossi, S.M. Parnell, W.W. Agace, Y. Takahama, E. J. Jenkinson, G. Anderson, Chemokine receptor expression defines heterogeneity in the earliest thymic migrants, Eur. J. Immunol. 37 (8) (2007) 2090–2096.

[37] U. Koch, E. Fiorini, R. Benedito, V. Besseyrias, K. Schuster-Gossler, M. Pierres, N. R. Manley, A. Duarte, H.R. Macdonald, F. Radtke, Delta-like 4 is the essential, nonredundant ligand for Notch1 during thymic T cell lineage commitment, J. Exp. Med. 205 (11) (2008) 2515–2523.

[38] J. Gommeaux, C. Grégoire, P. Nguessan, M. Richelme, M. Malissen, S. Guerder, B. Malissen, A. Carrier, Thymus-specific serine protease regulates positive selection of a subset of CD4+ thymocytes, Eur. J. Immunol. 39 (4) (2009) 956–964.

[39] I. Ohigashi, M. Kozai, Y. Takahama, Development and developmental potential of cortical thymic epithelial cells, Immunol. Rev. 271 (1) (2016) 10–22.

[40] N.L. Alves, O. Richard-Le Goff, N.D. Huntington, A.P. Sousa, V.S. Ribeiro, A. Bordack, F.L. Vives, L. Peduto, A. Chidgey, A. Cumano, R. Boyd, G. Eberl, J.P. Di Santo, Characterization of the thymic IL-7 niche in vivo, Proc. Natl. Acad. Sci. U. S. A. 106 (5) (2009) 1512–1517.

[41] Y. Fujimoto, L. Tu, A.S. Miller, C. Bock, M. Fujimoto, C. Doyle, D.A. Steeber, T. F. Tedder, CD83 expression influences CD4+ T cell development in the thymus, Cell 108 (6) (2002) 755–767.

[42] S. Shakib, G.E. Desanti, W.E. Jenkinson, S.M. Parnell, E.J. Jenkinson, G. Anderson, Checkpoints in the development of thymic cortical epithelial cells, J. Immunol. 182 (1) (2009) 130–137.

[43] A.R. Ribeiro, C. Meireles, P.M. Rodrigues, N.L. Alves, Intermediate expression of CCRL1 reveals novel subpopulations of medullary thymic epithelial cells that emerge in the postnatal thymus, Eur. J. Immunol. 44 (10) (2014) 2918–2924.

[44] F. Klein, C. Veiga-Villauriz, A. Börsch, S. Maio, S. Palmer, F. Dhalla, A.E. Handel, S. Zuklys, I. Calvo-Asensio, L. Musette, M.E. Deadman, A.J. White, B. Lucas, G. Anderson, G.A. Holländer, Combined multidimensional single-cell protein and RNA profiling dissects the cellular and functional heterogeneity of thymic epithelial cells, Nat. Commun. 14 (1) (2023) 4071.

[45] Y. Nakagawa, I. Ohigashi, T. Nitta, M. Sakata, K. Tanaka, S. Murata, O. Kanagawa, Y. Takahama, Thymic nurse cells provide microenvironment for secondary T cell receptor α rearrangement in cortical thymocytes, Proc. Natl. Acad. Sci. U. S. A. 109 (50) (2012) 20572–20577.

[46] H. Wekerle, U.P. Ketelsen, Thymic nurse cells–Ia-bearing epithelium involved in T-lymphocyte differentiation? Nature 283 (5745) (1980) 402–404.

[47] N. Lopes, A. Sergé, P. Ferrier, M. Irla, Thymic crosstalk coordinates medulla organization and T-cell tolerance induction, Front. Immunol. 6 (2015) 365.

[48] W. van Ewijk, E.W. Shores, A. Singer, Crosstalk in the mouse thymus, Immunol. Today 15 (5) (1994) 214–217.

[49] C. Meireles, A.R. Ribeiro, R.D. Pinto, C. Leitão, P.M. Rodrigues, N.L. Alves, Thymic crosstalk restrains the pool of cortical thymic epithelial cells with progenitor properties, Eur. J. Immunol. 47 (6) (2017) 958–969.

[50] N. Lopes, N. Boucherit, J.C. Santamaria, N. Provin, J. Charaix, P. Ferrier, M. Giraud, M. Irla, Thymocytes trigger self-antigen-controlling pathways in immature medullary thymic epithelial stages, Elife 11 (2022).

[51] T. Ueno, F. Saito, D.H. Gray, S. Kuse, K. Hieshima, H. Nakano, T. Kakiuchi, M. Lipp, R.L. Boyd, Y. Takahama, CCR7 signals are essential for cortex-medulla migration of developing thymocytes, J. Exp. Med. 200 (4) (2004) 493–505.

[52] T. Nitta, S. Nitta, Y. Lei, M. Lipp, Y. Takahama, CCR7-mediated migration of developing thymocytes to the medulla is essential for negative selection to tissue-restricted antigens, Proc. Natl. Acad. Sci. U. S. A. 106 (40) (2009) 17129–17133.

[53] M. Nasreen, T. Ueno, F. Saito, Y. Takahama, In vivo treatment of class II MHC-deficient mice with anti-TCR antibody restores the generation of circulating CD4 T cells and optimal architecture of thymic medulla, J. Immunol. 171 (7) (2003) 3394–3400.

[54] J. Gäbler, J. Arnold, B. Kyewski, Promiscuous gene expression and the developmental dynamics of medullary thymic epithelial cells, Eur. J. Immunol. 37 (12) (2007) 3363–3372.

[55] S.W. Rossi, M.Y. Kim, A. Leibbrandt, S.M. Parnell, W.E. Jenkinson, S.H. Glanville, F.M. McConnell, H.S. Scott, J.M. Penninger, E.J. Jenkinson, P.J. Lane, G. Anderson, RANK signals from CD4(+)3(-) inducer cells regulate development of Aire-expressing epithelial cells in the thymic medulla, J. Exp. Med. 204 (6) (2007) 1267–1272.

[56] T. Akiyama, Y. Shimo, H. Yanai, J. Qin, D. Ohshima, Y. Maruyama, Y. Asaumi, J. Kitazawa, H. Takayanagi, J.M. Penninger, M. Matsumoto, T. Nitta, Y. Takahama, J. Inoue, The tumor necrosis factor family receptors RANK and CD40 cooperatively establish the thymic medullary microenvironment and self-tolerance, Immunity 29 (3) (2008) 423–437.

[57] M. Irla, S. Hugues, J. Gill, T. Nitta, Y. Hikosaka, I.R. Williams, F.X. Hubert, H. S. Scott, Y. Takahama, G.A. Holländer, W. Reith, Autoantigen-specific interactions with CD4+ thymocytes control mature medullary thymic epithelial cell cellularity, Immunity 29 (3) (2008) 451–463.

[58] J.A. Williams, J. Zhang, H. Jeon, T. Nitta, I. Ohigashi, D. Klug, M.J. Kruhlak, B. Choudhury, S.O. Sharrow, L. Granger, A. Adams, M.A. Eckhaus, S.R. Jenkinson, E.R. Richie, R.E. Gress, Y. Takahama, R.J. Hodes, Thymic medullary epithelium and thymocyte self-tolerance require cooperation between CD28-CD80/86 and CD40-CD40L costimulatory pathways, J. Immunol. 192 (2) (2014) 630–640.

[59] Y. Hikosaka, T. Nitta, I. Ohigashi, K. Yano, N. Ishimaru, Y. Hayashi, M. Matsumoto, K. Matsuo, J.M. Penninger, H. Takayanagi, Y. Yokota, H. Yamada, Y. Yoshikai, J. Inoue, T. Akiyama, Y. Takahama, The cytokine RANKL produced by positively selected thymocytes fosters medullary thymic epithelial cells that express autoimmune regulator, Immunity 29 (3) (2008) 438–450.

[60] K.L. Wells, C.N. Miller, A.R. Gschwind, W. Wei, J.D. Phipps, M.S. Anderson, L. M. Steinmetz, Combined transient ablation and single-cell RNA-sequencing reveals the development of medullary thymic epithelial cells, Elife 9 (2020).

[61] F. Dhalla, J. Baran-Gale, S. Maio, L. Chappell, G.A. Holländer, C.P. Ponting, Biologically indeterminate yet ordered promiscuous gene expression in single medullary thymic epithelial cells, EMBO J. 39 (1) (2020) e101828.

[62] P. Brennecke, A. Reyes, S. Pinto, K. Rattay, M. Nguyen, R. Küchler, W. Huber, B. Kyewski, L.M. Steinmetz, Single-cell transcriptome analysis reveals coordinated ectopic gene-expression patterns in medullary thymic epithelial cells, Nat. Immunol. 16 (9) (2015) 933–941.

[63] L. Onder, V. Nindl, E. Scandella, Q. Chai, H.W. Cheng, S. Caviezel-Firner, M. Novkovic, D. Bomze, R. Maier, F. Mair, B. Ledermann, B. Becher, A. Waisman, B. Ludewig, Alternative NF-κB signaling regulates mTEC differentiation from podoplanin-expressing precursors in the cortico-medullary junction, Eur. J. Immunol. 45 (8) (2015) 2218–2231.

[64] R.J. Miragaia, X. Zhang, T. Gomes, V. Svensson, T. Ilicic, J. Henriksson, G. Kar, T. Lönnberg, Single-cell RNA-sequencing resolves self-antigen expression during mTEC development, Sci. Rep. 8 (1) (2018) 685.

[65] E. Lkhagvasuren, M. Sakata, I. Ohigashi, Y. Takahama, Lymphotoxin β receptor regulates the development of CCL21-expressing subset of postnatal medullary thymic epithelial cells, J. Immunol. 190 (10) (2013) 5110–5117.

[66] A. Ucar, O. Ucar, P. Klug, S. Matt, F. Brunk, T.G. Hofmann, B. Kyewski, Adult thymus contains FoxN1(-) epithelial stem cells that are bipotent for medullary and cortical thymic epithelial lineages, Immunity 41 (2) (2014) 257–269.
[67] S. Pinto, C. Michel, H. Schmidt-Glenewinkel, N. Harder, K. Rohr, S. Wild, B. Brors, B. Kyewski, Overlapping gene coexpression patterns in human medullary thymic epithelial cells generate self-antigen diversity, Proc. Natl. Acad. Sci. U. S. A. 110 (37) (2013) E3497–E3505.
[68] M. Matsumoto, H. Yoshida, K. Tsuneyama, T. Oya, Revisiting Aire and tissue-restricted antigens at single-cell resolution, Front. Immunol. 14 (2023) 1176450.
[69] Y. Hamazaki, M. Sekai, N. Minato, Medullary thymic epithelial stem cells: role in thymic epithelial cell maintenance and thymic involution, Immunol. Rev. 271 (1) (2016) 38–55.
[70] I.S. Khan, M.L. Mouchess, M.L. Zhu, B. Conley, K.J. Fasano, Y. Hou, L. Fong, M. A. Su, M.S. Anderson, Enhancement of an anti-tumor immune response by transient blockade of central T cell tolerance, J. Exp. Med. 211 (5) (2014) 761–768.
[71] D.H. Gray, N. Seach, T. Ueno, M.K. Milton, A. Liston, A.M. Lew, C.C. Goodnow, R. L. Boyd, Developmental kinetics, turnover, and stimulatory capacity of thymic epithelial cells, Blood 108 (12) (2006) 3777–3785.
[72] E. Bitoun, A. Micheloni, L. Lamant, C. Bonnart, A. Tartaglia-Polcini, C. Cobbold, T. Al Saati, F. Mariotti, J. Mazereeuw-Hautier, F. Boralevi, D. Hohl, J. Harper, C. Bodemer, M. D'Alessio, A. Hovnanian, LEKTI proteolytic processing in human primary keratinocytes, tissue distribution and defective expression in Netherton syndrome, Hum. Mol. Genet. 12 (19) (2003) 2417–2430.
[73] R.L. Van de Velde, N.B. Friedman, The thymic "myoidzellen" and myasthenia gravis, JAMA 198 (3) (1966) 287–288.
[74] J.E. Park, R.A. Botting, C. Domínguez Conde, D.M. Popescu, M. Lavaert, D.J. Kunz, I. Goh, E. Stephenson, R. Ragazzini, E. Tuck, A. Wilbrey-Clark, K. Roberts, V. R. Kedlian, J.R. Ferdinand, X. He, S. Webb, D. Maunder, N. Vandamme, K. T. Mahbubani, S.A. Teichmann, A cell atlas of human thymic development defines T cell repertoire formation, Science 367 (6480) (2020).
[75] V. Haunerdinger, M.D. Moccia, L. Opitz, S. Vavassori, H. Dave, M.M. Hauri-Hohl, Novel combination of surface markers for the reliable and comprehensive identification of human thymic epithelial cells by flow cytometry: quantitation and transcriptional characterization of thymic stroma in a pediatric cohort, Front. Immunol. 12 (2021) 740047.
[76] A.M. Farley, L.X. Morris, E. Vroegindeweij, M.L. Depreter, H. Vaidya, F. H. Stenhouse, S.R. Tomlinson, R.A. Anderson, T. Cupedo, J.J. Cornelissen, C. C. Blackburn, Dynamics of thymus organogenesis and colonization in early human development, Development 140 (9) (2013) 2015–2026.
[77] A. Finnegan, R.J. Cho, A. Luu, P. Harirchian, J. Lee, J.B. Cheng, J.S. Song, Single-cell transcriptomics reveals spatial and temporal turnover of keratinocyte differentiation regulators, Front. Genet. 10 (2019) 775.
[78] I. Van de Walle, E. Waegemans, J. De Medts, G. De Smet, M. De Smedt, S. Snauwaert, B. Vandekerckhove, T. Kerre, G. Leclercq, J. Plum, T. Gridley, T. Wang, U. Koch, F. Radtke, T. Taghon, Specific notch receptor-ligand interactions control human TCR-αβ/γδ development by inducing differential Notch signal strength, J. Exp. Med. 210 (4) (2013) 683–697.
[79] A. Lepletier, M.L. Hun, M.V. Hammett, K. Wong, H. Naeem, M. Hedger, K. Loveland, A.P. Chidgey, Interplay between follistatin, activin A, and BMP4 signaling regulates postnatal thymic epithelial progenitor cell differentiation during aging, Cell Rep. 27 (13) (2019) 3887–3901. .e4.
[80] C. Bonnans, J. Chou, Z. Werb, Remodelling the extracellular matrix in development and disease, Nat. Rev. Mol. Cell Biol. 15 (12) (2014) 786–801.
[81] A. Marx, J.K.C. Chan, L. Chalabreysse, S. Dacic, F. Detterbeck, C.A. French, J. L. Hornick, H. Inagaki, D. Jain, A.J. Lazar, M. Marino, E.M. Marom, A.L. Moreira, A.G. Nicholson, M. Noguchi, D. Nonaka, M.G. Papotti, S. Porubsky, L.M. Sholl, P. Ströbel, The 2021 WHO classification of tumors of the thymus and mediastinum: What is new in thymic epithelial, germ cell, and mesenchymal tumors? J. Thorac. Oncol. 17 (2) (2022) 200–213.
[82] Y. Yasumizu, N. Ohkura, H. Murata, M. Kinoshita, S. Funaki, S. Nojima, K. Kido, M. Kohara, D. Motooka, D. Okuzaki, S. Suganami, E. Takeuchi, Y. Nakamura, Y. Takeshima, M. Arai, S. Tada, M. Okumura, E. Morii, Y. Shintani, H. Mochizuki, Myasthenia gravis-specific aberrant neuromuscular gene expression by medullary thymic epithelial cells in thymoma, Nat. Commun. 13 (1) (2022) 4230.
[83] M. Radovich, C.R. Pickering, I. Felau, G. Ha, H. Zhang, H. Jo, K.A. Hoadley, P. Anur, J. Zhang, M. McLellan, R. Bowlby, T. Matthew, L. Danilova, A.M. Hegde, J. Kim, M.D.M. Leiserson, G. Sethi, C. Lu, M. Ryan, C.G.A. Network, The integrated genomic landscape of thymic epithelial tumors, Cancer Cell 33 (2) (2018) 244–258. .e10.
[84] E. Pardini, F. Cucchiara, S. Palumbo, G. Tarrini, A. Di Vita, F. Coppedè, V. Nicolì, M. Guida, M. Maestri, R. Ricciardi, V. Aprile, M.C. Ambrogi, S. Barachini, M. Lucchi, I. Petrini, Somatic mutations of thymic epithelial tumors with myasthenia gravis, Front. Oncol. 13 (2023) 1224491.
[85] I. Petrini, P.S. Meltzer, I.K. Kim, M. Lucchi, K.S. Park, G. Fontanini, J. Gao, P. A. Zucali, F. Calabrese, A. Favaretto, F. Rea, J. Rodriguez-Canales, R.L. Walker, M. Pineda, Y.J. Zhu, C. Lau, K.J. Killian, S. Bilke, D. Voeller, G. Giaccone, A specific missense mutation in GTF2I occurs at high frequency in thymic epithelial tumors, Nat. Genet. 46 (8) (2014) 844–849.
[86] O.B. Giorgetti, A. Nusser, T. Boehm, Human thymoma-associated mutation of the GTF2I transcription factor impairs thymic epithelial progenitor differentiation in mice, Commun. Biol. 5 (1) (2022) 1037.
[87] Y. He, I.K. Kim, J. Bian, A. Polyzos, D.C. Di Giammartino, Y.W. Zhang, J. Luo, M. O. Hernandez, N. Kedei, M. Cam, A.C. Borczuk, T. Lee, Y. Han, E.A. Conner, M. Wong, D.C. Tillo, S. Umemura, V. Chen, L. Ruan, G. Giaccone, A knock-in mouse model of thymoma with the GTF2I L424H mutation, J. Thorac. Oncol. (2022).