



Reframing thymic epithelial tumors through single-cell transcriptomics: a narrative review

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Background and Objective: Thymic epithelial tumors (TETs) are rare neoplasms originating from thymic epithelial cells (TECs). They exhibit histological, molecular, and immunological diversity shaped by aberrant interactions between neoplastic TECs and immune cells. World Health Organization (WHO) histological classification effectively captures their clinicopathological features, whereas inter- and intra-subtype heterogeneity persists. In this point, single-cell transcriptomics is well-suited to resolve cellular heterogeneity in TETs. This narrative review synthesizes findings from recent single-cell studies that illuminate epithelial subpopulations, intra-tumoral immune landscape, and disease-specific aberrations such as myasthenia gravis (MG)-associated phenotypes in TETs.

Methods: We conducted a focused narrative review on three key studies that applied single-cell RNA sequencing (scRNA-seq) to primary human TETs.

Key Content and Findings: Single-cell analyses identified a distinct TEC subpopulation ectopically expressing neuromuscular antigens, termed neuromuscular medullary TECs (nmTECs), in MG-associated thymomas, implicating aberrant antigen presentation and immune cell recruitment in autoantibody production. Other studies delineated tumor epithelial programs that shape the immune microenvironment, linking TEC transcriptional identities to the persistence of immature T-cells or dominance of mature T-cells. Bioinformatic approaches of ligand-receptor networks supported pathogenic crosstalk between neoplastic TECs and immune cells. Notably, independent groups converged on functionally oriented classifications that align across studies, stratifying TETs by epithelial lineage programs and immune composition with correspondence to biological behavior and prognosis.

Conclusions: Single-cell approaches have revealed TET heterogeneity encompassing abnormal epithelial cell states, immune development trajectories, and autoimmune-associated molecular programs with high resolution. The ongoing accumulation of single-cell transcriptomics datasets would refine a cell-centric framework for TET classification and pathogenesis, while also deepening insight into normal TEC biology.

Keywords: Thymic epithelial tumor (TET); thymoma; thymic carcinoma (TC); single-cell RNA-seq; myasthenia gravis (MG)

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Introduction

The thymus is a primary lymphoid organ that orchestrates the education of T-cells, enabling them to effectively eliminate foreign pathogens while simultaneously maintaining tolerance towards self-components. Developing T-cells in the thymus (i.e., thymocytes) progress through the double-negative (DN), the double-positive (DP), and finally the single-positive (SP) stages. During this procedure, thymic epithelial cells (TECs) in the cortical and medullary region distinctly play essential roles (1,2). Cortical TECs (cTECs) promote positive selection by sending survival signals to immature T-cells that recognize major histocompatibility complex (MHC) molecules (3). Subsequently, medullary TECs (mTECs) mediate negative selection to eliminate self-reactive T-cells through promiscuous self-antigen expression driven by the autoimmune regulator (AIRE) (4-7). These compartmentalized epithelial programs remain foundational to our understanding of T-cell development.

Thymic epithelial tumors (TETs) are rare neoplasms originating from TECs, yet they represent the most common tumors of the adult anterior mediastinum. In the current World Health Organization (WHO) classification, they are divided into six main subtypes of thymomas [types A, AB, B1–B3, and micronodular thymoma with lymphoid stroma (MNT)] and thymic carcinoma (TC), within an integrated diagnostic framework that incorporates clinical, morphological, immunophenotypic, and molecular features (8,9). This histological framework effectively captures the clinicopathological features of TETs, including their prognosis and association with autoimmunity—most notably myasthenia gravis (MG). Nevertheless, considerable heterogeneity exists even within a single histological subtype, making TETs challenging to classify reproducibly (10). Even within individual TETs, cellular heterogeneity across the tumor is prominent; for example, type B1 thymomas exhibit a cortex-like region rich in immature T-cells admixed with scattered medullary islands. Immunohistochemical studies support that cTEC/mTEC programs (e.g., $\beta 5t$ /PRSS16/cathepsin V *vs.* claudin-4/CD40/AIRE) are variably recapitulated across subtypes (11-14).

A large-scale multi-omics profiling by The Cancer Genome Atlas (TCGA) projects has significantly advanced our understanding of TETs (15). Furthermore, TCGA supports the robustness of the current WHO classification, while still revealing some degree of intra-subtype heterogeneity. Several studies have leveraged

transcriptomics data in TCGA to delineate molecular characteristics of TETs (13,16,17) and their association with MG (18-20). However, the paucity of tumor epithelial cells embedded within dense immune cells—particularly in thymomas—can obscure tumor-intrinsic signals in bulk assays. In this context, single-cell technologies offer substantial advantages for capturing transcriptional signatures from rare cellular subsets, such as TEC-derived tumor cells. Indeed, single-cell studies conducted in normal TECs have revealed extensive TEC heterogeneity and developmental trajectories in mice (21-34) and humans (35-39) that provide essential reference maps for interpreting their neoplastic states.

In this narrative review, we summarize recent single-cell transcriptomic studies that have proposed redefined TET classification, immune-epithelial crosstalk, and the clinicopathological spectrum, including a work that links epithelial programs to MG pathogenesis (40-42). We present this article in accordance with the Narrative Review reporting checklist (available at <https://med.amegroups.com/article/view/10.21037/med-2025-1-45/rc>).

Methods

We searched PubMed for original studies applying single-cell RNA sequencing (scRNA-seq) to the primary TETs, including thymomas and TCs (in particular thymic squamous cell carcinoma), using combinations of the search terms “thymic epithelial tumor”, “thymoma”, “thymic carcinoma”, and “single-cell RNA-seq”. The full search strategy is shown in *Table 1*. We excluded review articles and studies limited to secondary analyses or re-analyses of previously published datasets.

Overview of single-cell studies in TETs

Based on the methodology outlined above, we selected three primary studies applying single-cell transcriptomics to human TETs. *Table 2* summarizes the cohort composition of each study. The following sections revisit their main findings, focusing on epithelial lineage states and immune contexture.

Identification of MG-associated mTEC subpopulation

The study employed an integrated analysis of bulk and scRNA-seq to elucidate the pathogenesis of thymoma-

Table 1 The search strategy summary

Items	Specification
Date of search	August 31st, 2025
Database searched	PubMed
Search terms used	“Thymic epithelial tumor”, “thymoma”, “thymic carcinoma”, “single-cell RNA-seq”
Timeframe	2022–2024
Inclusion and exclusion criteria	Original articles applying single-cell RNA sequencing to the primary thymic epithelial tumors were included. We excluded review articles and studies limited to secondary analyses or re-analyses of previously published datasets
Selection process	All authors independently reviewed all articles selected according to the predefined inclusion and exclusion criteria

Table 2 Cohort composition of previously reported single-cell studies relating to TETs

Author	Sample (in-house)	Autoimmunity
Yasumizu <i>et al.</i> (41) [2022]	AB (n=2), B1 (n=1), B2 (n=1)	All patients positive for anti-AChR Ab (no seronegative controls)
Xin <i>et al.</i> (40) [2022]	A (n=1), AB (n=2), B3 (n=1), MNT (n=1), TC (n=1)	One patient (AB) accompanying MG
Liu <i>et al.</i> (42) [2024]	AB (n=3), B1 (n=1), B2 (n=1), MT (n=1), TC (n=1)	No information available

A, type A thymoma; AB, type AB thymoma; anti-AChR Ab, anti-acetylcholine receptor antibody; B1, type B1 thymoma; B2, type B2 thymoma; B3, type B3 thymoma; MG, myasthenia gravis; MNT, micronodular thymoma with lymphoid stroma; MT, metaplastic thymoma; TC, thymic carcinoma; TET, thymic epithelial tumor.

associated MG (TAMG). Initially, by analyzing bulk RNA-seq data from the TCGA archive, the authors identified a unique expression signature of neuromuscular-associated molecules such as GABRA5 in TAMG. This gene expression signature, revealed by weighted gene co-expression network analysis (WGCNA) (43), was designated as the “yellow module”. The yellow module was also characterized by high expression of specific keratins, including KRT6, KRT15, and KRT32. The study also reported a slight increase in the expression of the acetylcholine receptor (AChR) gene, CHRNA1, a primary autoantigen in MG.

Subsequently, scRNA-seq was performed on thymoma tissue and peripheral blood mononuclear cells from anti-AChR antibody-positive thymoma patients. This analysis led to the identification of a distinct subpopulation of mTECs, termed neuromuscular mTECs (nmTECs), which ectopically express these neuromuscular molecules. These nmTECs were found to have high expression of both MHC class I and II molecules, suggesting a high capacity for antigen presentation. Furthermore, the study provided a detailed analysis of the immune microenvironment within TAMG, suggesting the formation of ectopic germinal

centers and B-cell maturation with the recruitment of T follicular helper cells. Predicted cell-cell interactions analysis (44) also identified a CXCL12-CXCR4 crosstalk between nmTECs and immune cells, which was suggested to be crucial for autoantibody production.

Finally, deconvolution analysis (45) of the TCGA bulk data, using the scRNA-seq-derived cell annotations, suggested that nmTECs represent the most significantly expanded cell population in TAMG. These findings propose a pathological model where nmTECs present ectopic self-antigens, leading to the abnormal activation of the immune system and subsequent MG pathogenesis. In their subsequent research, authors demonstrated that these nmTECs are predominantly localized at the cortico-medullary junction using spatial transcriptomics (46).

Alternative classification of TETs based on the immune landscape

The authors utilized a multi-faceted approach, including mass cytometry (CyTOF), scRNA-seq, and T cell receptor (TCR) repertoire analysis, to decode the immune landscape

of TETs. The study proposes a new molecular classification system that divides TETs into three subtypes—type 1 (T1), type 2 (T2), and type 3 (T3)—based on the developmental patterns of intra-tumoral immune cells. This new classification did not align perfectly with conventional WHO classifications or Masaoka stages but further captured the prognosis. Each subtype is characterized by a unique set of tumor cell characteristics and its corresponding impact on T-cell development and the tumor immune microenvironment:

- ❖ T1: this subtype is defined by a substantial infiltration of immature T-cells, particularly DP T-cells. T-cell development is shown to be arrested at an early stage, with Notch and Wnt signaling pathways being highly active. The tumor cells are characterized as KRT14⁺/GNB3⁺ TECs with low expression levels of human leukocyte antigen-DR (HLA-DR), which may impair the positive selection of T-cells.
- ❖ T2: similar to T1, T2 tumors exhibit a high abundance of immature T-cells, but T-cell development is actively promoted. Single-cell analysis revealed that DP cells in T2 tumors highly express genes associated with positive selection, such as those related to the NF- κ B signaling pathway. Additionally, genes involved in IL-7 signaling, which drives intra-thymic T-cell expansion, are highly expressed. The tumor cells comprise CCL25⁺ cTEC-like cells, which are likely to facilitate positive selection.
- ❖ T3: this subtype is characterized by a notable absence of immature T-cells, with a predominance of mature SP T-cells, including an increased proportion of regulatory T-cells (Tregs). The epithelial tumor cells are identified as CHI3L1⁺ mTEC-like cells that seem to provide minimal support for T-cell development. Instead, the tumor microenvironment is dominated by tissue-resident memory (T_{RM}) T-cells, particularly CD8⁺ T_{RM} cells, suggesting immune recruitment from the periphery induced by malignant cells. These CD8⁺ T_{RM} cells, expressing high levels of CD39, IFN- γ , CXCR3, and CXCL13, are suggested to be activated through interactions with the tumor cells, driving the immune response and the recruitment of other immune cells.

Finally, the genes GNB3 and CHI3L1 are highlighted as potential prognostic biomarkers for the newly defined

TET subtypes T1 and T3, respectively. This study presents a comprehensive framework that connects the distinct epithelial origins of each subtype to specific T-cell developmental trajectories and unique immune microenvironments.

Dissecting the heterogeneity in TETs and their abnormal cellular states

Integrating scRNA-seq data from in-house TET samples with normal TECs (35,36) and TETs from the Xin dataset (40), the authors constructed a large-scale single-cell atlas to overcome the limitations of conventional histopathological classification, proposing a new framework that links specific cellular populations to tumor heterogeneity and prognosis. The study identified KRT14⁺ progenitor-like cells as a key epithelial population, which exhibit a spindle-shaped morphology and are enriched in thymomas. Based on the presence or absence of these cells, the authors reclassified TETs into three subtypes: T1 (KRT14⁺ thymoma), T2 (KRT14⁻ thymoma), and T3 (TC; KRT14⁻).

- ❖ T1: this subtype is characterized by a high proportion of KRT14⁺ progenitor-like cells with prominent infiltration of immature DP T-cells. Their signature as the progenitor was supported by the directional flow from KRT14⁺ cells to other mTECs/cTECs in the RNA velocity analysis (47,48), and the highest scores for both “early progenitor” and “postnatal progenitor” signatures [gene sets identified by a mouse TEC study (29)] among all epithelial cells. In the TCGA cohort, the T1 group showed a significantly better prognosis compared to other subtypes, suggesting that KRT14 may serve as a positive prognostic biomarker for TETs.
- ❖ T2: this subtype is defined by a low expression of KRT14 and a prominent infiltration of immature DP T-cells, similar to the T1 subtype, but with distinct molecular characteristics. T2 thymomas are associated with the accumulation of PRSS16⁺ cTEC-like population and are suggested to maintain the immature T-cell population through interactions such as the CCL25-CCR9 axis. These T-cells are in an abnormal developmental state, showing augmented expression of RAG1/RAG2 and uncoordinated expression of TCR rearrangement genes. This developmental error is attributed to

a lack of CCL21-expressing mTECs, which are essential for guiding immature T-cells from the cortex to the medulla for proper maturation.

- ❖ T3: this subtype corresponds to TCs and is marked by high expression of MSLN, CCL20, and SLC1A5 in tumor cells. The immune microenvironment is dominated by mature SP T-cells, particularly CD8⁺ T-cells that overexpress effector molecule granzyme B (GZMB) together with immune checkpoint molecules such as LAG3 and HAVCR2. The study also identified a unique macrophage-based metabolic pattern where macrophages supply glutamine to malignant cells via the LGALS9-SLC1A5 axis, promoting tumor progression. This metabolic crosstalk was linked to a poor prognosis.

Furthermore, the research identified a rare, abnormal population of FOXI1⁺ ionocytes, a specialized epithelial cell type dedicated to transepithelial ion transport. These cells co-expressed ASCL3, KRT7, and V-ATPase but showed low expression of CFTR, a canonical marker of ionocytes. These neoplastic ionocytes are hypothesized to originate from KRT14⁺ progenitor-like cells because they share a common HRAS missense mutation and demonstrate a close evolutionary relationship in the trajectory analysis. These cells were also shown to interact with macrophages via the RARRES2-CMKLR1 axis, suggesting a potential “energy dialogue” within the tumor microenvironment.

In summary, Liu *et al.* (42) provide a large-scale single-cell atlas that redefines TET subtypes based on cellular composition, identifies novel biomarkers, and reveals crucial cell-cell communication pathways.

Integrative perspective of TETs from single-cell studies

Collectively, these pioneering single-cell studies provide a high-resolution map of the cellular ecosystems within TETs based on functional cellular states. Xin *et al.* and Liu *et al.* independently proposed a tripartite classification scheme (T1, T2, and T3) that offers potential prognostic stratification. This framework anchors TET subtypes to distinct epithelial cell programs that fundamentally shape the immune contexture, particularly the state of intra-thymic T-cell development. Intriguingly, despite different analytical starting points, the proposed subtypes show strong conceptual alignment and are broadly consistent with the conventional WHO classification. The subtype

associated with a favorable prognosis (T1) is consistently defined by variable amounts of KRT14⁺ epithelial cells and immature DP T-cells. This subtype is mainly dominated by the type A and AB thymomas in the conventional histological classification. A second thymoma subtype (T2) is characterized by the predominance of cTEC-like tumor cells and a distinct T-cell developmental defect, proposed by both studies. The T2 mainly comprises type B1/B2 thymomas, followed by type AB thymomas, consistent with a cortex-like region in their histology. Finally, both classifications converge on TC (T3) as a separate entity defined by the absence of active thymopoiesis and an infiltrate of mature SP T-cells, such as effector CD8⁺ T-cells and FOXP3⁺ Tregs. The integrative framework of these classification systems is visually summarized in *Figure 1*.

These emerging results suggest that the biological identity of TETs is, in part, dictated by which normal TEC program the tumor cells recapitulate. The work by Yasumizu *et al.* (41) enriches this model by providing a mechanistic link between a specific, aberrant epithelial state and MG. They demonstrated how a unique subpopulation, nmTECs, can hijack the antigen-presentation machinery to ectopically express self-antigens, thereby driving the autoimmune pathogenesis (*Figure 1*).

Conclusions

The application of single-cell transcriptomics has deepened our understanding of TETs, complementing a morphology-based classification with a more functionally relevant, cell-centric framework. Single-cell technologies are ideal methods to dissect heterogeneous populations, such as TETs, revealing a complex ecosystem of interacting epithelial and immune cells that was previously obscured in bulk analyses or morphology alone. Indeed, by anchoring neoplastic TEC states to normal TEC biology at single-cell resolution, scRNA-seq studies begin to elucidate the fundamental biology of thymic epithelial tumorigenesis. The accumulation of scRNA-seq studies in TECs has demonstrated that KRT14⁺ TECs exhibit immature TEC characteristics, and this immature TEC signature emerges as a key epithelial program in the T1 subtype in TETs. Furthermore, Liu *et al.* have shown that in T3, tumor progression appears to be driven not only by the intrinsic properties of CHI3L1⁺ mTEC-like tumor cells, but also by their interaction with macrophages. In particular, metabolic crosstalk via the LGALS9-SLC1A5 axis has been experimentally shown to promote the malignant growth

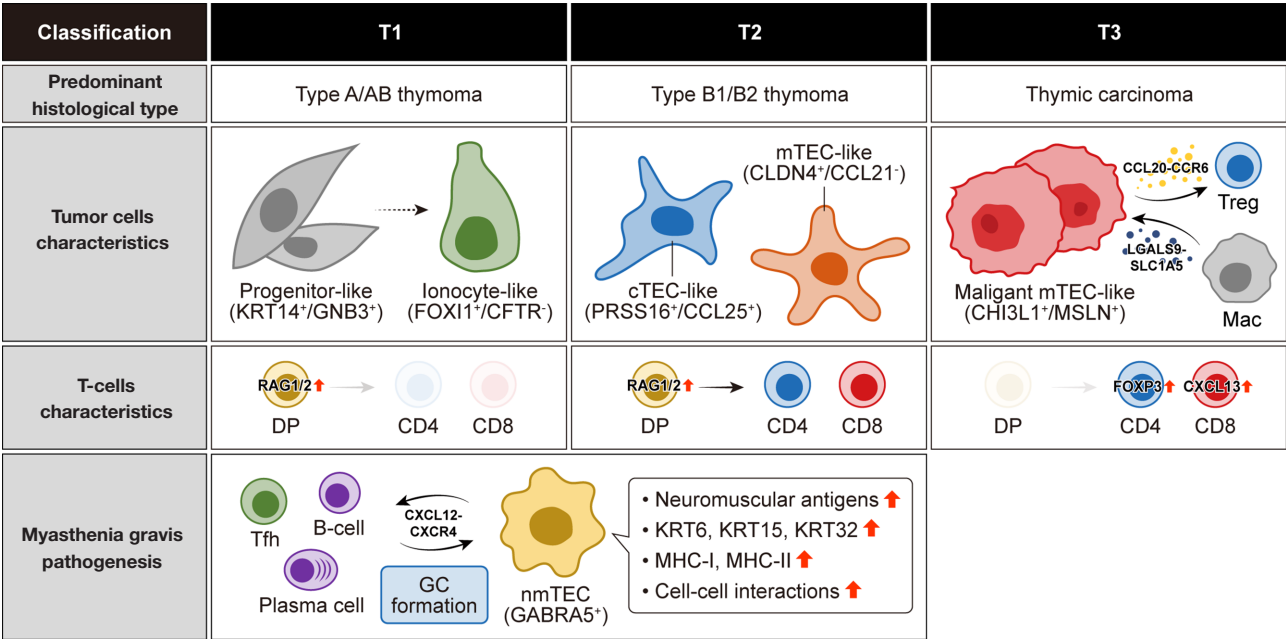


Figure 1 Integrative perspective of TETs from single-cell studies. Integrative framework of classification systems proposed by single-cell studies, shown alongside a single-cell-based model of myasthenia gravis pathogenesis. CD4, CD4⁺ T-cell; CD8, CD8⁺ T-cell; cTEC, cortical thymic epithelial cell; DP, double-positive T-cell; GC, germinal center; Mac, macrophage; MHC, major histocompatibility complex; mTEC, medullary thymic epithelial cell; nmTEC, neuromuscular mTEC; Tfh, T follicular helper cell; TET, thymic epithelial tumor; Treg, regulatory T-cell.

of TC organoids. Because the LGALS9-SLC1A5 has also been implicated in several other tumor types (49,50), where it is associated with invasive behavior and worse prognosis, it offers a plausible mechanistic explanation for the aggressive clinical behavior of TC. Additionally, ongoing efforts to inhibit SLC1A5-dependent glutamine metabolism (51) highlight this axis as an attractive direction for future therapeutic development. Building on the WHO classification, the proposed T1, T2, and T3 subtypes refine our understanding of TET biology by mapping conventional histological categories onto distinct TEC differentiation trajectories and associated immune contexts, biological behavior, and MG, while single-cell studies further delineate the underlying TEC molecular subtypes and transcriptional programs.

Despite these significant advances, several limitations and future directions must be acknowledged. The sample sizes in these initial studies are relatively small, and future work with larger, more diverse cohorts is necessary to validate the proposed classifications. For instance, type B3 thymoma (represented by only one sample) was classified as T3 in the study by Xin *et al.*, whereas the same case was categorized

as T2 (KRT14⁺ thymoma) according to the criteria used by Liu *et al.* Moreover, rare entities such as MNT and metaplastic thymoma (MT) remain difficult to generalize within this framework. Although the overall conceptual frameworks proposed by these groups are broadly aligned, such discrepancies highlight gaps in the current evidence and underscore the need for further data and consensus-building before a robust molecular classification can be established. Further research is also needed to clarify the histopathological specificity of unique subsets, such as the nmTECs, and their prevalence across different subtypes. Since the scRNA-seq analysis is only applied to anti-AChR antibody-positive thymoma patients, the inclusion of seronegative controls would further strengthen the authors' conclusions.

Methodological challenges also warrant consideration. Given the scarcity of TECs within a dense lymphoid stroma, enrichment steps are often required prior to scRNA-seq. Furthermore, the limited sensitivity of droplet-based sequencing platforms might fail to capture lowly expressed but functionally critical genes. Another plate-based approach (52,53) would provide much higher sensitivity

and near-complete full-length transcript coverage. The advent of a spatial transcriptomics platform that can simultaneously achieve high sensitivity and high resolution is eagerly awaited to overcome these limitations and restore the crucial spatial context lost during tissue dissociation. This approach will allow for the *in situ* mapping of cell-cell interactions, such as the dialogue between tumor cells and immune cells, providing a more complete picture of the tumor microenvironment.

In summary, the study of neoplastic TECs offers a unique opportunity for a bidirectional understanding of normal and pathological states. Not only does our knowledge of normal thymic biology inform our interpretation of TETs, but the analysis of their aberrant developmental programs may, in turn, shed new light on the fundamental mechanisms of normal TEC biology and T-cell education. Studying these disrupted TEC systems can reveal the importance of regulatory mechanisms that are difficult to discern in the normal state. These foundational single-cell studies pave the way for a more refined molecular classification of TETs, which will hopefully translate into improved diagnostic accuracy and novel therapeutic strategies tailored to the specific biology of each tumor subtype.

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Footnote

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aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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