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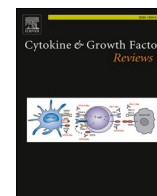
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Commentary

Tightly bound T-cell clusters: A new class of hyper-effector tumor killers

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ABSTRACT

T cells have traditionally been characterized as autonomous killers that eliminate malignant cells through single-cell cytotoxicity. However, recent findings reveal that tumor-reactive T cells frequently form tightly bound clusters on the tumor surface, functioning as cooperative hyper-effector units. These clusters establish stable and reinforced immune synapses, sustain prolonged engagement with tumor targets, and coordinate the delivery of perforin, granzymes, and proinflammatory cytokines. As a result, they exhibit markedly enhanced tumor-killing capacity compared with unbound T cells in both ex vivo assays and murine models. This discovery introduces an essential spatial and mechanical dimension to cancer immunology and challenges the prevailing assumption that molecular phenotype alone defines T-cell efficacy. The implications are substantial, extending to tumor-infiltrating lymphocyte (TIL) therapy optimization, the refinement of immunotherapy biomarkers, and the engineering of next-generation CAR-T and CAR-NK platforms. Enriching or mimicking the behavior of cluster-forming T cells may accelerate clinical responses and overcome barriers associated with solid tumors. Collectively, these insights reposition T-cell clustering as a fundamental determinant of effective antitumor immunity.

1. Introduction

T cells are central to antitumor immunity and are traditionally viewed as individual effector units that recognize and destroy malignant cells through antigen-specific cytotoxicity. However, recent mechanistic work from the Netherlands Cancer Institute has challenged this single-cell paradigm by demonstrating that tumor-reactive T cells frequently organize into tightly bound multicellular clusters that adhere directly to tumor cells and to one another [1]. These clusters function as hyper-efficient killing units distinguished by enhanced synapse stability, cooperative granule secretion, and amplified cytokine signaling. Similar to recent conceptual shifts surrounding NK-cell regulatory roles in checkpoint immunotherapy [2], these findings highlight the importance of spatial and mechanical organization in shaping immune function within the tumor microenvironment. Fig. 1 illustrates this emerging model, showing how clustered T cells coordinate cytotoxic activity more effectively than isolated T cells, resulting in accelerated tumor destruction.

2. Cooperative cytotoxicity beyond single cell killing

The formative observation of this research is that clustered T cells remain stably attached to tumor cells for extended periods, enabling highly efficient and coordinated cytotoxic responses. Compared with solitary T cells, cluster-forming T cells produce stronger and more sustained immune synapses, concentrate their cytolytic granules more effectively at the tumor interface, and maintain superior effector function in suppressive microenvironments [1]. The cooperative interaction among clustered T cells creates a functional synergy that significantly increases killing efficiency. This behavior suggests the emergence of a spatially defined subset of hyper-effector T cells whose activity cannot be fully explained by conventional molecular phenotypes or

transcriptional markers.

3. Implications for tumor-infiltrating lymphocyte (TIL) therapy and functional T-cell selection

The discovery of cluster-forming T cells has immediate relevance for improving TIL-based therapies. Current strategies rely heavily on phenotypic markers such as PD-1, CD39, and CD103, or on transcriptomic and cytokine profiles, all of which provide indirect proxies for cytotoxic potential [3]. By contrast, physical clustering represents a direct, function-first indicator of tumor engagement and killing competence. Isolating these cluster-forming T cells from patient tumors could significantly enhance the potency and consistency of TIL products. Moreover, cluster formation may correlate with critical differentiation trajectories, particularly the transition from stem-like progenitor exhausted T cell (Tpex) states to terminally exhausted cytotoxic T cell (Ttex) populations that are associated with durable therapeutic responses [4–6]. Thus, cluster-based selection strategies may streamline TIL manufacturing and improve therapeutic outcomes.

4. Predictive biomarker potential in checkpoint blockade

The spatial organization of T cells within the tumor microenvironment (TME) may hold predictive value for responsiveness to immune checkpoint blockade [7]. Traditional biomarkers such as PD-L1 expression, tumor mutational burden (TMB), or infiltration scores do not directly assess whether T cells are physically engaging tumor cells. In contrast, the prevalence, density, and stability of tightly bound T-cell clusters offer a more physiologically grounded indicator of effective antigen recognition and synapse formation [8]. Incorporating cluster-associated metrics into spatial transcriptomics, multiplex imaging, and artificial intelligence (AI)-driven digital pathology platforms

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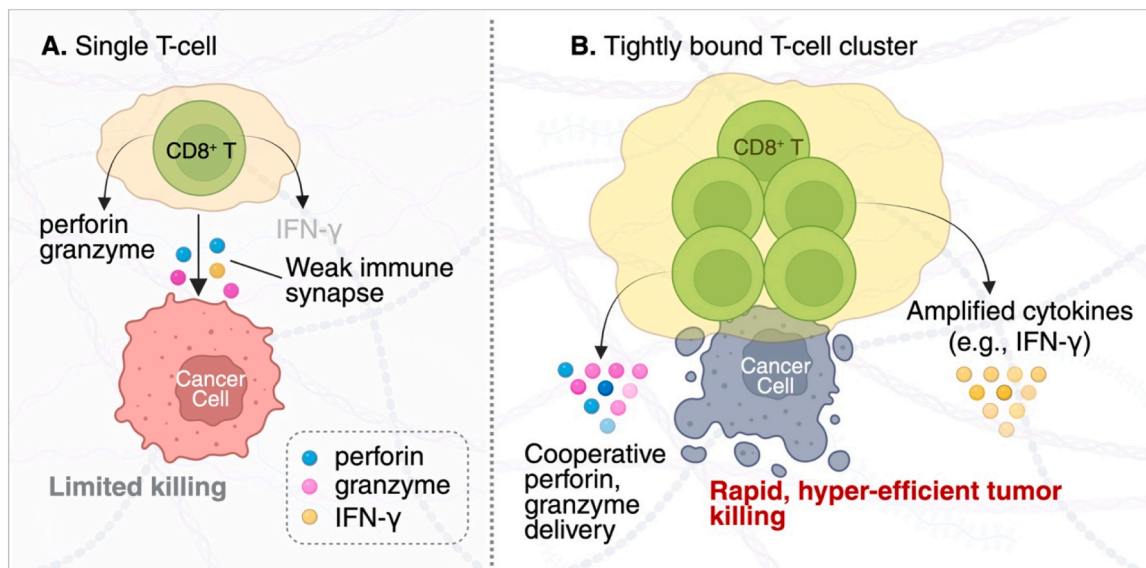


Fig. 1. Cooperative cytotoxicity mediated by tightly bound T-cell clusters. (A) Single CD8⁺ T cells form weak, inefficient immune synapses with cancer cells, resulting in limited delivery of perforin and granzyme, low IFN- γ production, and slow, incomplete tumor killing. (B) In contrast, tightly bound multicellular T-cell clusters generate reinforced and stable synapses that support coordinated perforin-granzyme release, amplified cytokine production (e.g., IFN- γ), and rapid, hyper-efficient tumor lysis. Cluster formation represents a functional state of heightened antigen engagement and may serve as a dynamic biomarker of cytotoxic potency and a potential therapeutic target to enhance anti-tumor immunity.

may significantly improve predictive accuracy for immunotherapy responsiveness [9]. These spatial biomarkers could complement and, in some cases, outperform conventional molecular markers by providing a real-time snapshot of functional immune engagement.

5. Relevance for engineered cell therapies

The principles underlying T-cell clustering extend directly to engineered immune cell therapies such as chimeric antigen receptor (CAR)-T, CAR-NK, and CAR-macrophage platforms [10]. Effective cytotoxicity in solid tumors depends not only on antigen recognition but also on the ability of engineered cells to maintain stable and durable contact with malignant targets despite mechanical resistance and immunosuppressive cues [11]. Cluster-forming behavior reflects the combined influence of adhesion molecules, cytoskeletal reinforcement, synaptic architecture, and local cytokine signaling. Engineering CAR constructs that strengthen adhesion, promote cooperative killing, or enhance synapse stability may significantly improve therapeutic activity in solid tumors, a major challenge for current CAR therapies [12]. Strategies that modulate the extracellular matrix or the structural properties of the TME may also facilitate cluster formation and increase immune cell dwell time at tumor sites.

6. A spatial and mechanical dimension to cancer immunology

The discovery of tightly bound T-cell clusters introduces a critical spatial dimension to cancer immunology. Tumors often display immune exclusion, where T cells accumulate at stromal margins without effectively infiltrating tumor nests. Even in tumors with abundant TILs, only a small subset of T cells may establish productive contact with malignant cells. Cluster-forming T cells appear capable of overcoming these spatial restrictions by maintaining stable interactions with tumor cells, resisting suppressive influences from myeloid populations, and sustaining cytotoxicity even when expressing exhaustion markers [13]. These observations underscore the importance of integrating spatial and mechanical biology into the analysis of T-cell function, complementing recent efforts to redefine NK-cell behavior within the TME [14].

7. Clinical and translational opportunities

The translational potential of T-cell clustering spans diagnostics, biomarker development, and therapeutic engineering. Diagnostic platforms such as microfluidic capture systems, live-cell imaging, and short-term ex vivo co-culture assays could be adapted to quantify cluster-forming T cells directly from patient tumor samples, offering rapid, function-based assessment of immune competence. From a biomarker perspective, the morphology, frequency, and stability of T-cell clusters could refine patient selection for checkpoint blockade or combination immunotherapies. In the context of cell therapy manufacturing, enriching for cluster-forming T cells may generate more potent therapeutic products with enhanced persistence and cytotoxicity. Engineering applications are equally promising, as CAR-T and CAR-NK constructs optimized to promote stable synapse formation and cooperative killing may overcome barriers that currently limit their efficacy in solid tumors [15].

8. Conclusion

The identification of tightly bound T-cell clusters as hyper-effector killing units represents a significant conceptual advance in cancer immunology. By shifting attention from individual T-cell phenotypes to their collective spatial and mechanical behavior, these findings provide a new framework for understanding effective tumor engagement. The implications stretch across TIL therapy optimization, immunotherapy biomarker development, and the engineering of next-generation immune cell therapies. As immuno-oncology continues to evolve, incorporating spatial and mechanical dimensions into immune profiling may be essential for improving therapeutic precision and clinical outcomes.

CRediT authorship contribution statement

Wenxue Ma: Conceptualization, Writing – original draft, Writing – review & editing, Finalization. **Catriona Jamieson:** Supervision, Writing – review & editing.

Ethics approval and consent to participate

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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