Intraepithelial ILC1-like cells: Front-line fighters in human head and neck squamous cell carcinoma

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Innate lymphoid cells (ILCs) are a heterogeneous population of tissue-associated lymphoid cells, particularly abundant in mucosal and barrier tissues, such as gut and skin, which survey their microenvironment by perceiving and rapidly responding to incoming cues. ILC heterogeneity is characterized by function, and while numerous soluble and cell-surface factors trigger ILC function, major subgroups among ILCs correspond to the cytokines they produce and can respond to. Major ILC subsets comprise ILC3s, ILC2s, and ILC1s, which are the corresponding counterpart of T helper 17 (Th17), Th2, and Th1/CD8 T cells, respectively (1, 2). Additional subsets in the literature correspond to specialized functions and ontogeny. ILCs are also characterized by a remarkable plasticity that allows them to adjust to evolving tissue stimuli and adapt to different tasks (2, 3). Therefore, intermediate ILC states exist in tissues, which harbor signatures of the different ILC major subgroups (4). ILCs have also been found in mouse and human tumors and their function and plasticity in the tumor microenvironment (TME) are now beginning to be elucidated (5, 6).

ILC1s are a further heterogeneous group as they include conventional natural killer (NK) cells and noncytolytic helper ILC1s as well as intraepithelial ILC1s (1, 2). ILC1s are well defined in mouse models by ontogeny and phenotype as NK1.1^{+} CD127^{+} Eomes^{-} Tbet^{+} cells (7, 8). However, in humans their nature, phenotype, and origin are still a matter of debate due to overlapping functional profiles and lack of similar well-defined ontogeny as in mice. In human peripheral blood, conventional NK cells have long been known to include CD56^{bright} and CD56^{dim} subsets, which specialize in cytokine production and cytotoxicity, respectively (9). In tissues and blood, helper noncytotoxic CD56^{-} CD127^{+} ILC1s were initially identified (10), but their identity was later called into question (11, 12). Intraepithelial ILC1s (ieILC1s) were

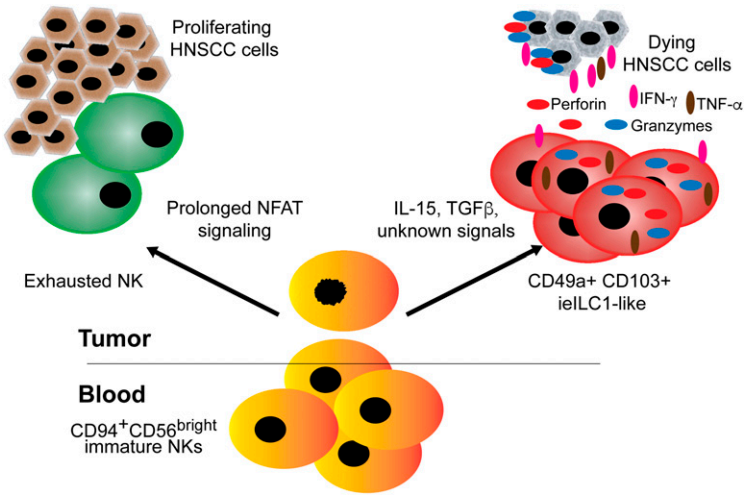

Fig. 1. Immature NK cells enter the tumor and depending on specific signaling encountered in the tissue microenvironment develop either into exhausted NKs that promote tumor dissemination and proliferation or into ieILC1-like cells with potent cytotoxic and cytokine secretion effector function.

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originally described in tonsils and the intraepithelial layer of the gut as $CD56^+$ $CD127^-$ $NKp44^+$ $CD103^+$ cells (13). Later the presence of $CD56^+$ $CD103^+$ $CD127^-$ ieILC1-like cells, with a variegated expression of NKp44, was reported in other pathological tissues, such omentum adipose tissue from obese patients, lung tumors, and colorectal tumors (11). However, because ieILC1s and ieILC1-like cells expressed Tbet and Eomes, and in some compartments had cytolytic mediators such as perforin and granzymes, their identity as true ILC1s and their relationship to conventional tissue-resident NK cells are still disputed (11, 13).

Moreno-Nieves et al. (14), in PNAS, identify in human head and neck squamous cell carcinoma (HNSCC) a cell type closely resembling ieILC1s, which exerts a potent antitumor activity in vitro, and in vivo, in NOD-*scid* IL2R-gamma^{null} (NSG) immunodeficient mice implanted with a human HNSCC tumor cell line. This cell type has some phenotypic and functional resemblance to a similar previously described $NK1.1^+$ $CD103^+$ $CD49a^+$ ILC1-like cell found in mouse models of MMTV-PyMT mammary tumors, which curbs tumor growth due to high cytotoxic potential (15).

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HNSCC cancers are a heterogeneous family of tumors arising from the oral cavity, pharynx, and larynx mucosal membranes (16). They are largely linked to cigarette smoking, alcohol consumption, or human papilloma virus (HPV)16 infection. Despite surgical intervention, radiotherapy, and chemotherapy, HNSCC frequently recurs and causes death of the patients affected (16, 17). This burden is substantial, considering that HSNCC is the sixth most common cancer worldwide. Recent advances have helped to understand the immune landscape in HSNCC and have indicated that $HPV16^+$ HSNCCs are heavily infiltrated by $CD8^+$ T cells that may recognize viral antigens. These $CD8^+$ T cells can be exploited therapeutically and reinvigorated with checkpoint blockade therapy to induce killing of cancer cells (18). Accordingly, the 5-y overall survival rate of patients affected by HPV^+ HSNCC is almost twofold better than the overall survival rate of patients presenting with HPV^- HSNCC (70 to 80% versus 40 to 60%).

NK cells were previously identified in HSNCC (19). However, their phenotypic and functional heterogeneity was still poorly understood. Moreno-Nieves et al. (14), in PNAS, leverage high-throughput single-cell RNA sequencing (scRNAseq) to identify NK cell and related ILC1 subsets from tumors of eight HSNCC cancer patients and compare them to blood-derived NK cells of two healthy donors. Moreno-Nieves et al. (14) identify seven clusters of tumor-associated cells that group separately from blood cytotoxic NKs (cluster 1). One of these clusters represents $RORC^+$ ILC3s (cluster 6). Based on the expression of CD127, CD62L, CD117, and CCR7, a second cluster (cluster 5) could represent either helper ILC1s or tissue-associated $CD56^{bright}$ NK cells. In addition, two distinct NK cell clusters emerge: NK1s (cluster 2) exhibit a strong interferon-induced signature, while NK2s (cluster 3) are characterized by expression of transcription factors such as NR4A1 and NR4A2, which have been shown to be associated with T cell exhaustion (20–22). In T cells, NR4A1 and NR4A2 are induced by chronic NFAT stimulation both in models of chronic viral infection and in cancer and are highly expressed in $CD8^+$ T cells bearing multiple inhibitory receptors, such as PD1 and TIM3 (21, 22). Whether the NK2 cluster of "exhausted" NK cells, identified by Moreno-Nieves et al. (14), behaves similarly to exhausted $CD8^+$ T cells by up-regulating multiple immune checkpoint inhibitors is not clear, possibly due to the relatively shallow number of genes detected by scRNAseq. Cluster 4 has intermediate features between the $CD127^+$ ILC1/$CD56^{bright}$ and the NK1/2 clusters, again supporting the notion that cells with intermediate features are also present in the TME, in addition to mucosal tissues (4).

The most striking observation emerging from the Moreno-Nieves et al. (14) study is the identification of two clusters (clusters 7 and 8) that have features of the previously identified ieILC1s including expression of CD49a, CD69, CD103, and the chemokine receptor CXCR6 (13). These cells are present in both HPV^+ and HPV^- cancers and, similar to ieILC1s, have cytotoxic mediators and express both Tbet and Eomes. Unexpectedly, ieILC1-like cells in HNSCC have potent antitumor activity both in vitro and in vivo and produce greater amounts of TNFα and IFNγ in response to tumor cells than conventional NKs. Moreover, one of the two ieILC1-like clusters (cluster 8) is actively proliferating in vivo, as indicated by Ki67 expression, suggesting that ieILC1-like cells actively expand in the TME. Moreno-Nieves et al. (14) further show that immature peripheral blood $CD94^+CD56^{bright}$ cells, when exposed to HNSCC cells in the presence of soluble IL-15, multiply and acquire CD103 and CD49a expression. This differentiation process requires cell–cell contact via yet unknown receptor–ligand interactions, as well as TGFβ signaling. Importantly, in The Cancer Genome Atlas databases of HNSCC cancers, IL-15 expression positively correlates with the ieILC1-like cell signature and negatively with the NK2 signature of exhausted, NR4A1/NR4A2-expressing NKs. TGFβ imprinting of ieILC1s in tonsil was previously suggested based on the original gene signature identified (13). However, a requirement for TGFβ to boost ILC1 responses to tumors is unexpected, given that TGFβ is generally viewed as an immunosuppressive cytokine. In addition, recent evidence shows that enhanced TGFβ signaling deviates cells from a NK cell phenotype, which has antitumor activity, to an ILC1 module, which promotes tumor growth and dissemination (23, 24). It is conceivable that in the HNSCC TME multiple and more complex interactions are taking place or that the TGFβ-imprinted signature is driven by other members of the TGFβ family, such as activin or BMPs. In addition, in the HNSCC TME the TGFβ activity may be tightly and temporally modulated, as shown for the skewing of Th17 to T regulatory T cell fates in the context of sustained TGFβ signaling (25). Alternatively, as hypothesized by Moreno-Nieves et al. (14), the immunostimulatory or immunosuppressive properties of TGFβ may vary and be context dependent. TGFβ signaling could boost ILC1 function when IL-15, or other yet unknown cytokines, is available in the same tissue-specific niche and/or when selective stimulatory or costimulatory cell surface receptors are engaged at the same time on immature precursor cells.

Overall, based on the pseudotime analysis of the NK/ILC clusters identified by scRNAseq in this study, Moreno-Nieves et al. (14) depict a scenario where an immature circulating NK cell, perhaps a $CD56^{bright}$ NK cell, enters the tumor and under the influence of cognate signals present in the TME becomes either an ieILC1-like cell, which will proliferate, expand, and fight the tumor with energy and resilience, or an exhausted NK cell, which will eventually give up and lose the battle (Fig. 1). Understanding how

to tip the balance in favor of one fate versus the other might be key to designing new strategies to manipulate immune cells in HNSCC to improve therapeutic options to this still frequently fatal and highly morbid disease.

1 L. Krabbendam, J. H. Bernink, H. Spits, Innate lymphoid cells: From helper to killer. *Curr. Opin. Immunol.* **68**, 28–33 (2021).
2 M. Colonna, Innate lymphoid cells: Diversity, plasticity, and unique functions in immunity. *Immunity* **48**, 1104–1117 (2018).
3 A. I. Lim, T. Verrier, C. A. Vosshenrich, J. P. Di Santo, Developmental options and functional plasticity of innate lymphoid cells. *Curr. Opin. Immunol.* **44**, 61–68 (2017).
4 M. Cella *et al.*, Subsets of ILC3-ILC1-like cells generate a diversity spectrum of innate lymphoid cells in human mucosal tissues. *Nat. Immunol.* **20**, 980–991 (2019).
5 A. Crinier *et al.*, Multidimensional molecular controls defining NK/ILC1 identity in cancers. *Semin. Immunol.* **30**, 101424 (2020).
6 T. Bald, M. Wagner, Y. Gao, S. Koyasu, M. J. Smyth, Hide and seek: Plasticity of innate lymphoid cells in cancer. *Semin. Immunol.* **41**, 101273 (2019).
7 J. Zhang *et al.*, T-bet and Eomes govern differentiation and function of mouse and human NK cells and ILC1. *Eur. J. Immunol.* **48**, 738–750 (2018).
8 A. P. McFarland *et al.*, Multi-tissue single-cell analysis deconstructs the complex programs of mouse natural killer and type 1 innate lymphoid cells in tissues and circulation. *Immunity* **54**, 1320–1337.e4 (2021).
9 A. G. Freud, B. L. Mundy-Bosse, J. Yu, M. A. Caligiuri, The broad spectrum of human natural killer cell diversity. *Immunity* **47**, 820–833 (2017).
10 J. H. Bernink *et al.*, Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. *Nat. Immunol.* **14**, 221–229 (2013).
11 Y. Simoni *et al.*, Human innate lymphoid cell subsets possess tissue-type based heterogeneity in phenotype and frequency. *Immunity* **48**, 1060 (2018).
12 J. H. Bernink, J. Mjösberg, H. Spits, Human ILC1: To be or not to be. *Immunity* **46**, 756–757 (2017).
13 A. Fuchs *et al.*, Intraepithelial type 1 innate lymphoid cells are a unique subset of IL-12- and IL-15-responsive IFN-γ-producing cells. *Immunity* **38**, 769–781 (2013).
14 U. Y. Moreno-Nieves *et al.*, Landscape of innate lymphoid cells in human head and neck cancer reveals divergent NK cell states in the tumor microenvironment. *Proc. Natl. Acad. Sci. U.S.A.*, 10.1073/pnas.2101169118 (2021).
15 S. Dadi *et al.*, Cancer immunosurveillance by tissue-resident innate lymphoid cells and innate-like T cells. *Cell* **164**, 365–377 (2016).
16 A. von Witzleben, C. Wang, S. Laban, N. Savelyeva, C. H. Ottensmeier, HNSCC: Tumour antigens and their targeting by immunotherapy. *Cells* **9**, E2103 (2020).
17 C. S. Farah, Molecular landscape of head and neck cancer and implications for therapy. *Ann. Transl. Med.* **9**, 915 (2021).
18 M. Canning *et al.*, Heterogeneity of the head and neck squamous cell carcinoma immune landscape and its impact on immunotherapy. *Front. Cell Dev. Biol.* **7**, 52 (2019).
19 A. J. Charap *et al.*, Landscape of natural killer cell activity in head and neck squamous cell carcinoma. *J. Immunother. Cancer* **8**, e001523 (2020).
20 H. Seo *et al.*, TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose $CD8^+$ T cell exhaustion. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 12410–12415 (2019).
21 J. Chen *et al.*, NR4A transcription factors limit CAR T cell function in solid tumours. *Nature* **567**, 530–534 (2019).
22 X. Liu *et al.*, Genome-wide analysis identifies NR4A1 as a key mediator of T cell dysfunction. *Nature* **567**, 525–529 (2019).
23 V. S. Cortez *et al.*, Transforming growth factor-β signaling guides the differentiation of innate lymphoid cells in salivary glands. *Immunity* **44**, 1127–1139 (2016).
24 Y. Gao *et al.*, Tumor immunoevasion by the conversion of effector NK cells into type 1 innate lymphoid cells. *Nat. Immunol.* **18**, 1004–1015 (2017).
25 I. I. Ivanov, L. Zhou, D. R. Littman, Transcriptional regulation of Th17 cell differentiation. *Semin. Immunol.* **19**, 409–417 (2007).