Single-Cell RNA Sequencing Reveals a Developmental Hierarchy in Langerhans Cell Histiocytosis

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Summary: In this issue of Cancer Discovery, Halbritter and colleagues utilize single-cell RNA sequencing to dissect the cellular hierarchy in Langerhans cell histiocytosis. They identified a remarkably consistent composition of 14 cellular subsets across all patients with a range of clinical spectrums consistent with a shared developmental hierarchy driven by key transcriptional regulators.

See related article by Halbritter et al., p. 1406 (7).

Histioytic disorders are a group of clonal myeloid diseases associated with somatic mutations in the MAPK pathway (1). The most frequent of these is Langerhans cell histiocytosis (LCH) where granulomatous lesions comprised of langerin-positive histiocytes and an inflammatory infiltrate arise in a multitude of organs including but not limited to bone, skin, liver, spleen, pituitary, and lungs. Clinical presentation varies significantly, ranging from a single lesion to multisystem disease. The demonstration that these lesions are clonal with the majority carrying activating BRAF mutations provides strong support for the concept that LCH is a neoplastic disease (2, 3). The cell from which this disease derives remains an active area of investigation. Previous studies on hematopoietic cells of pediatric patients with low-risk and high-risk disease demonstrated the presence of the BRAFV600E mutation in circulating mononuclear cells only in patients with high-risk multisystem disease (4). In contrast, BRAFV600E was absent in the peripheral blood of patients with low-risk single-lesion disease and was restricted to the LCH lesions themselves. A subset of high-risk patients also carried BRAFV600E in the CD34+ hematopoietic progenitor compartment, establishing a model whereby the acquisition of BRAFV600E in stem cells leads to high-risk disease as opposed to a committed tissue-resident dendritic cell precursor that causes low-risk single-lesion disease. A very rare subtype of congenital self-healing LCH is limited to skin involvement that spontaneously resolves (5). Because tissue-resident macrophages including Langerhans cells in the developing fetus are first derived from a transient hematopoiesis wave in the yolk sac during development, this raises the possibility of a fetal yolk sac cell of origin in a subset of patients. Consistent with this hypothesis, a recent study introducing BRAFV600E into yolk sac erythromyeloid progenitors (EMP) of mice led to clonal expansion of tissue-resident macrophages followed by late-onset neurodegenerative disorder, a complication of LCH that occurs in a small subset of patients (6). Furthermore, evaluation of brain biopsies in patients with histiocytic disorders that suffered from neurodegenerative disease demonstrated the presence of BRAFV600E in the microglia of these patients at the sites of neuronal loss. The authors therefore propose EMPs as a potential cell of origin in patients with this devastating complication.

To further understand the clinical heterogeneity, cellular nature, and underlying pathophysiology of LCH, Halbritter and colleagues subjected 7 LCH lesions from patients with different levels of involvement (low-risk single-system and multisystem disease) and from different tissue sites (lymph node, skin, bone) to single-cell RNA sequencing (scRNA-seq; ref. 7). Transcriptional profiles shared across all cell subsets and all patients identified a gene signature consistent with previously published LCH bulk transcriptome datasets. Moving down to single-cell resolution revealed a developmental hierarchy of progenitors and progeny that was surprisingly consistent among all specimens despite differences in sample characteristics, albeit with varying frequencies of each population. Fourteen specific subsets were bioinformatically inferred that differed in their levels of single-cell transcriptional entropy, a discriminator of pluripotent and nonpluripotent cell types, forming a gradient of cells with decreasing entropy corresponding to a more restricted transcriptional profile and differentiated cell state (Fig. 1; ref. 8). To confirm their clonal origin, high-entropy and low-entropy subsets were evaluated for the presence of BRAFV600E and found to have consistent mutational rates of 85% to 90%, similar to the bulk population. The highest-entropy, least-differentiated cellular subsets had elevated levels of genes involved in cell proliferation including DNA replication and cell cycle-related genes. In contrast, low-entropy subsets carried transcriptional profiles consistent with more differentiated states, including the LCH-S11 and LCH-S12 populations that expressed markers of mature Langerhans cells and dendritic cells. Of the maturing cells, two subsets (LCH-S13 and LCH-S14) had elevated levels of transcripts linked to destructive inflammatory behavior, such as genes associated with the IFN response and osteoclast differentiation. This
suggests they are involved in osteolysis and tissue destruction, a clinical feature seen in patients which is often attributed to being secondary to an inflammatory infiltrate as opposed to derived from the neoplastic cells themselves. Overall, the data are consistent with progenitor-like cells giving rise to maturing cells resembling epidermal Langerhans cells and dendritic cells as well as aggressive cells that are inflammatory in nature. An integrated network-based analysis of transcriptome, chromatin profiling via the assay for transposase-accessible chromatin using sequencing (ATAC-seq), and transcription factor-binding sites was then undertaken to visualize the regulatory programs within the different subsets. The authors identified gene-regulatory hubs shared across all subsets such as the hematopoietic transcription factor SPI1, as well as subset-specific networks, for example, the MYBL2 gene that was unique to the LCH-S1 progenitor subset and is known to be involved in cancer initiation and cell proliferation. The LCH-S12 dendritic cell subset was uniquely dominated by IRF8, an IFN-dependent mediator of dendritic cell function and differentiation. Combined, their data support a shared developmental hierarchy at the single-cell level with a progenitor population giving rise to more differentiated states driven by key transcriptional regulators.

These studies are consistent with an emerging theme from single-cell datasets whereby phenotypically similar populations are in fact comprised of heterogeneous populations. In the traditional view of hematopoiesis, cells were proposed to differentiate through discrete sets of bifurcations producing distinct populations with decreasing lineage potential and self-renewal activity. Hematopoietic malignancies that result from mutations that cause a so-called “differentiation block” were previously taught to result in a maturational arrest that was a hard stop, for example, cells were unable to terminally differentiate. These two concepts of clonality and maturational arrest are now being challenged as sophisticated next-generation sequencing technology and bioinformatic analyses allow the field to visualize cells with increasing levels of granularity. Genomic studies were among the first to break this model, demonstrating the presence of oligoclonality at the DNA level. Consistent with emerging data in acute myelogenous leukemia...

Figure 1. Origin of LCH. Sites of fetal hematopoiesis throughout gestation are shown. Microglia and the earliest Langerhans cells (LC) are derived from yolk sac progenitors. Later in gestation, definitive hematopoiesis arises from the bone marrow. Definitive hematopoietic stem cells (HSC) give rise to dendritic cell precursors (DCP). Yolk sac progenitors, HSC, and DCP have all been proposed as the cell of origin for LCH following the acquisition of somatic activating MAPK mutations. Single-cell RNA sequencing of LCH lesions demonstrates significant heterogeneity with 14 cellular subsets that display a gradient of entropy consistent with progenitor and more differentiated states. LCH-S1 and LCH-S2 populations have a gene-expression profile consistent with high levels of proliferation. These cells presumably maintain the neoplasm. LCH-S11 and LCH-S12 have evidence of a more differentiated state, expressing elevated levels of Langerhans cell and dendritic cell markers. LCH-S13 cells express genes associated with the IFN response, suggesting they contribute to the inflammation seen in this entity. Varying proportions of subpopulations may explain the clinical spectrum seen. For example, patients with aggressive multisystem disease may have greater numbers of LCH-S1 and LCH-S2 cells, whereas those with low-risk single lesions may be predominantly comprised of LCH-S11 and LCH-S12 populations. Similarly, patients with a significant inflammatory component may have greater amounts of LCH-S13 and LCH-S1. AGM, aorta-gonad-mesonephros.
and other hematopoietic malignancies, Halbritter and colleagues have now shown that LCH populations transformed with a uniform driver retain the ability for maturation to a certain extent, leading to subpopulations that have characteristics of cells at varying stages of differentiation (9). The differentiation block is, in essence, only partial. This suggests the requirement of a pool of so-called “cancer stem cells” that can persist in the absence of differentiation to maintain the malignancy, a concept supported by functional xenograft studies in other malignant models. The data, however, leave unanswered questions, many of which require in vivo and in vitro model systems: To what degree are cells that proceed along this differentiation spectrum committed as opposed to retaining plasticity to change from one subset to another? Are all populations able to give rise to the full spectrum of populations? How does one reconcile this data with the prior studies suggesting multisystem and single-lesion diseases are derived from cells with different maturational status? Do central nervous system lesions carry the same composition of populations? Do differences in the frequency of LCH cell subsets reflect their clinical behavior in terms of presentation and response to therapy? Is this hierarchy seen in other histiocytic disorders such as Erdheim-Chester disease and juvenile xanthogranuloma? All of these questions remain under active investigation, and the information we learn from forthcoming studies will allow us to not only understand the biology of this fascinating disease, but improve upon the current clinical outcomes as well.

In summary, Halbritter and colleagues provide compelling evidence that patients with LCH share a developmental hierarchy at the single-cell level, suggesting these patients may have a common hematopoietic cell of origin.

Disclosure of Potential Conflicts of Interest

T.A. Gruber is a consultant at Bristol-Myers Squibb. No other potential conflicts of interest were disclosed.

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