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STEM CELLS PLAY A ROLE IN LEUKEMIA FROM THE BEGINNING TO THE END

Although cancer is often studied as bulk tissue, it is clear that a tumor is not simply a bag of functionally homogeneous cells. Individual cells of a tumour can have variation in many of the hallmarks of cancer including growth, death, metabolism, and other properties and these heterogeneous properties contribute to therapy evasion, disease progression and relapse.

We are interested in a related question: *is every cancer cell equally able to keep propagating the tumor or is this property restricted to a limited set of cancer cells* akin to many normal tissue hierarchies where only stem cells have the capacity for self-renewal that allows them to sustain tissue regeneration long term?

Decades of accumulated evidence had supported the idea that the regenerative potential of individual cells in a tumour are highly variable and not all tumour cells are able to persist over the long term. Early studies of the 1960s using classic pulse-chase experiments where acute leukemia patients were infused with tritiated thymidine showed that most blasts were not cycling but postmitotic. They were constantly replenished from a smaller proportion (<10%) of proliferating cells. Two proliferative fractions were found: a more abundant faster cycling subset that could be labeled within 24-hour cycle time and a smaller fraction of slower cycling cells with a dormancy time that could last weeks to months.

This result was quite unexpected and predicted that *anti-proliferation therapies that formed the core of hitherto chemotherapy regimens would only target actively cycling cells but spare the more dormant cells*; such patients were doomed to relapse.

A new assay for proliferating leukemia cells was developed around that time that measured that only 1/100 cells had the ability to make colonies in soft agar. Then Jim Griffin showed that the colony was composed of CD33+ cells but that it sometimes was initiated from CD33- cells. Together, these studies gave strong evidence that *not every* **acute myeloid leukemia (AML)** *cell was the same.* But what was not clear is whether AML was a cellular hierarchy composed of distinct populations (dormant, proliferative, or non-proliferating) or whether leukemia cells were in fact equal but could somehow transition between any of these proliferative states.

Our own studies of cellular hierarchies came from the study the normal human blood system, *where we developed the first system for transplanting human hematopoietic cells into immune-deficient mice with resultant multilineage repopulation*. We then extended this to human lymphoid leukemia (B-ALL), AML and chronic myeloid leukemia (CML). The xenograft assay is now the “gold standard” for detecting human hematopoietic stem cells (HSCs) and **leukemic stem cells (LSCs).**

The key test of whether AML was a hierarchy or whether all cells were equal came when we purified AML into four distinct subpopulations based on the staining of stem-cell associated antigens and then used the xenograft assay to test each fraction for leukemia-initiating activity; if all cell fractions generated AML, then all cells were equal. However, we found that only one fraction initiated leukemia in xenografts and all four fractions were recreated again.

Serial transplantation of that fraction was a key test of long-term propagation and self-renewal capacity; proof that this was an LSC. Thus, *AML is hierarchically organized and sustained by self-renewing LSCs*.

LSC specific gene signatures were developed and then through machine learning of clinical datasets, we showed that they were more predictive of patient response and outcome than non-LSC leukemia cells.

This has led to the development of a robust stemness-based prognostic/predictive test for AML that is currently being tested clinically. We and others have begun to **develop new drugs that target LSCs** with the goal of ensuring that when AML is treated to remove the bulk blasts that the surviving LSC would also be eradicated.

LSC represent a non-genetic description of heterogeneity but there is also overwhelming evidence from advanced genome technology that cancer within a single patient is a heterogeneous mixture of genetically distinct subclones that reflect complex mutational evolution. In an attempt to harmonize these two concepts of heterogeneity, we asked whether there were genetically diverse LSC that drive each subclone.

We carried out comprehensive analysis of diagnosis and relapse samples in AML and ALL using xenografting. We showed that LSC were genetically diverse at diagnosis and we could find rare LSC-driven subclones already fated to cause relapse. This means that the seeds of relapse have already evolved long before diagnosis and are lying buried within rare LSC. The evidence that relapse in patients is so closely tied to the properties of LSC provided key proof of their relevance. We showed that relapse-fated subclones are drug tolerant and possess distinct metabolic and stemness programs that allow them to both survive therapy and regenerate relapse disease. These pathways offer new classes of therapeutic targets that could be used in a novel strategy to identify and target relapse-fated subclones already at diagnosis before they can evolve further causing leukemia relapse.

Serendipitously, these combined genomic and stem cell studies of diagnostic leukemia samples led us to uncover the earliest stages of leukemia. A diagnostic blood sample contains large numbers of leukemia cells but about 10-20% of normal blood cells remain. Instead of being completely normal, we found these cells contained one of the same mutations as carried by leukemia cells. This work established that the non-leukemia cells came from a mutated HSC that was also the ancestor of the AML cells; these are termed **pre-leukemic HSC** and gave insight that HSC represent the cellular origin of AML.

This raised the important question of *how long before diagnosis did the disease originate* and more importantly can such pre-leukemic individuals be identified in the general population before the AML arises. We investigated enrolment blood samples from large population cohorts and found evidence of pre-leukemic HSC only in those people who eventually developed AML over the next decade.

The identification of individuals at risk for progression to AML sets the stage for future strategies aimed at preventing AML. These studies elucidated the full arc of leukemia from the cell of origin, the initiating mutation, the initial clonal expansion, the creation of genetically diverse LSC and finally the origin of relapse initiating cells.

Broadly, our studies have shown that leukemias, need to be studied functionally at the single cell level and that the *long-term propagating LSC are key to understanding whether a cell will respond to therapy or whether it will survive therapy and ultimately cause relapse*.

These findings provide an opportunity for improved clinical monitoring of AML patients and ALL patients and the development of therapies to prevent disease occurrence and/or to prevent progression to relapse.

More broadly, our studies in leukemia have been replicated for some **solid tumours** with the identification of cancer stem cells and suggesting the concept of a tumor cell hierarchy might underlie many cancer types.