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Decision-Making Circuitry of Blood Stem Cells Mapped

Experimental work has led to the first mathematical model of a regulatory circuit that blood-forming stem cells use to decide what kind of white blood cell they will become. The model explains the puzzling behavior of differentiating stem cells, in which they simultaneously assume genetic signatures of different cell types before committing to one identity, said the researchers who developed it.

The researchers said that the concepts used to assemble their circuit diagram could enable stem cell biologists to induce immature stem cells to develop into specific types of cells for therapeutic purposes. Although they conducted their studies with blood-forming, or hematopoietic, stem cells, the researchers said the basic principles of the regulatory network likely also govern cell type determination in other tissues, such as the brain and intestine. The researchers also said their findings could offer insight into leukemias, in which “indecision” in the regulatory circuitry seems to drive cancerous proliferation.

The researchers, led by Howard Hughes Medical Institute investigator Harinder Singh, published their findings in the August 25, 2006, issue of the journal *Cell*. Singh and his experimental team, including Peter Laslo, who spearheaded the project, are at the University of Chicago. For the mathematical modeling, the team collaborated with University of Chicago colleague Aaron Dinner.

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- Harinder Singh

The researchers focused on two types of white blood cells, known as macrophages and neutrophils. Macrophages are the long-lived garbage disposals of the immune system, indiscriminately engulfing and digesting cellular debris and pathogens. The shorter-lived neutrophils are the immune system's vultures, flocking to the site of an infection to target and ingest invading organisms. Each cell type relies on its own set of functionally active genes to carry out its particular role in fighting infection.

Before they reach maturity, however, macrophages and neutrophils—and the genes they express—are identical. Both come from cells known as myeloid progenitors. What Singh and his colleagues wanted to know was how a myeloid progenitor cell decides whether to become a macrophage or a neutrophil.

The regulatory system of these cells consists basically of proteins called transcription factors that control the activity of many genes -- which in turn comprise the molecular machinery that instructs cells to develop into their mature, functioning state.

A major scientific puzzle, said Singh, has been how and why immature hematopoietic stem cells initially express genes that are characteristic of more than one cell lineage. "One can imagine that the cells are molecularly previewing their developmental options by turning on at low levels cell-type-specific genes, and thereby revealing the developmental potential that they have," said Singh.

Understanding the circuitry that controls this critical "transcriptional priming" is central to understanding how different kinds of stem cells develop and what kind of developmental potential they have, said Singh. Unfortunately, he said, there had not been an experimental cell system that researchers could manipulate to explore that circuitry. To fill that need, Singh

and his colleagues used findings from their lab and others to develop such a system. Their system was based on earlier studies in which Singh and his colleagues identified a transcription factor called PU.1 as a central genetic switch that triggers development of myeloid progenitor cells. Other researchers had identified a transcription factor called C/EBP α as another development trigger.

However, said Singh, how these two factors govern stem cell differentiation was a mystery. “Both macrophages and neutrophils have very high levels of PU.1 and C/EBP α ,” he said. “They both appear to directly activate a mix of both macrophage- and neutrophil-specific genes. Yet in macrophages, the neutrophil genes are off, and in neutrophils the macrophage genes are off.

“So the conundrum is how a shared set of primary regulators acting on these macrophage and neutrophil genes could nevertheless produce a specific pattern of gene expression in each of the two cell types. In other words, why don't PU.1 and C/EBP α inappropriately activate neutrophil genes in the context of a macrophage, and vice versa?”

The researchers used mouse progenitor cells lacking PU.1 to explore this mystery. They then manipulated those cells' decision-making machinery by introducing different amounts of PU.1.

When the researchers introduced low concentration of PU.1, they found that the cells activated both macrophage and neutrophil genes. “This was highly significant, because it showed for the first time how this mixed lineage pattern is set up,” said Singh.

The researchers found that when they increased the concentration of PU.1, which induced macrophage differentiation, the cells first went through a transitory mixed lineage state, but then triggered new regulatory proteins that repressed neutrophil genes and activated macrophage genes. The researchers also identified a counteracting repressor protein in the cells that actively shut off macrophage-favoring genes during neutrophil differentiation.

According to Singh, the discovery of the counteracting repression circuitry is likely key to understanding stem cell regulation in general. “We think that if this property of mixed lineage transcriptional priming is shared amongst different kinds of stem cells and multipotential progenitors, then resolving these mixed lineage states will invariably involve counteracting repressors,” he said.

From these and other experiments with genetically altered mouse progenitor cells, Singh and his colleagues formulated a mathematical model that depicts the regulatory network governing progenitor cell development. “Our network has the property that when there are low levels of the primary regulators -- PU.1 or C/EBP α -- there is a mixed lineage pattern of gene expression,” said Singh. “As we arbitrarily increase one of the two regulators, we have built

into the model the counteracting regulatory circuitry that causes the genetic pattern to begin to resolve in one developmental direction or the other.”

According to Singh, their model could help explain how cells in many tissues or organs can develop using either instructive regulatory signals, as well as via non-instructive or random regulatory programs in which the cells become “bi-stable” before randomly developing into one cell type or another.

Such insights could have important implications for the clinical use of stem cells to rejuvenate tissues damaged by disease or trauma, said Singh. “There is a lot of excitement in stem cell biology these days about the possibility of rationally and efficiently generating particular cell types from different stem cells for therapeutic purposes,” he said. “As we better understand the underlying genetic circuitry that orchestrates development of a particular kind of stem cell into a specialized cell type, we should be able to manipulate it for such purposes.”

Understanding of leukemias also could be aided by insight into the hematopoietic stem cell regulatory circuitry, said Singh. “It’s been a puzzle that lots of leukemias exhibit mixed lineage patterns of gene expression - for example of both macrophage and lymphocyte genes,” he said. “It may be that these cells are stuck in this progenitor-like state, and if you could induce them to resolve that state -- that is, differentiate into one or the other cell types -- they would cease to be tumorigenic.”