Stanford School of Medicine
NEWS RELEASES
NOVEMBER 5, 2008

Stanford research sheds light on key trigger of embryonic stem cell differentiation

BY KRISTA CONGER

Clusters of mouse embryonic stem cells called embryoid bodies more closely approximate true embryos in organization and structure than previously thought, according to researchers at the Stanford University School of Medicine. Harnessing the signals that influence the cells' fate may help researchers more accurately direct the differentiation of embryonic stem cells for use in therapy.

The researchers found that embryoid bodies have hallmarks of gastrulation - a remarkable developmental step that launches a hollow ball of cells toward becoming an organism with three distinct types of precursor cells. The scientists showed that this process is initiated by a single signaling pathway in embryoid bodies and in real embryos. Enhancing or blocking this signal affects what the cells become, the scientists found.

"A lot of embryonic stem cell research is aimed at devising ways to help the cells differentiate along a particular path," said Roeland Nusse, PhD, professor of developmental biology. "But it's very difficult to know how to do this. We're learning that they do more things in culture than we previously thought; at the same time, we're developing more tools to control what they become."

Nusse is the senior author of the research, published in the Nov. 6 issue of the journal Cell Stem Cell. He is also a Howard Hughes Medical Institute investigator and a member of Stanford's Cancer Center. The study was funded in part by a grant from the California Institute of Regenerative Medicine intended to clarify the role of a common group of cell signaling molecules called the Wnt family in the differentiation of embryonic stem cells.

Nusse and the first authors of the paper, postdoctoral scholar Derk ten Berge, PhD, and undergraduate student Wouter Koole, used easily tracked reporter genes that are expressed only when cells are responding to Wnt signals to figure out when and where Wnt is active in mouse embryos and embryoid bodies. Embryoid bodies are clumps of embryonic stem cells that can to begin to differentiate into different tissues but they are not true embryos.

Using this system, the researchers learned that Wnt-responsive cells first appear in 6.5-day-old embryos in an area called the primitive streak that forms on what will become the posterior side of the embryo. It is the first step toward gastrulation, in which an outer layer of cells dimples inward at what will be either the mouth or anus to form the three distinct precursor cell types shared by most animals: the ectoderm, or outer layer, which forms neurons, skin cells and pigment; the endoderm, or inner layer, which forms many of the organs; and mesoderm, or middle layer, which forms muscle and red blood cells.

More importantly, Nusse and his colleagues determined that Wnt-responsive cells in the embryoid bodies also spontaneously form a primitive streak, though they never truly gastrulate. Supplementing the naturally occurring Wnt signal with "extra" Wnt protein accelerated the formation of the primitive streak, and adding proteins that blocked Wnt activity inhibited it.

"We knew that embryoid bodies did exhibit some self-organization," said Nusse. "They form a hollow cavity with inner and outer cell layers. But the primitive streak is the first indication we have that they can develop the kind of asymmetry that is seen in embryos."

Furthermore, the extra Wnt caused the Wnt-responsive cells to differentiate primarily into mesendodermal precursors (which can become either mesoderm or endoderm and is associated with the posterior of the embryo) and inhibited the formation of neurectoderm (ectoderm destined to become cells of the nervous system that are mostly associated with the embryo's anterior). Blocking Wnt activity tipped the balance in the other direction, causing the cells to shun mesendoderm and become mainly neurectoderm.

The first step to controlling cell fate is to understand which protein in the normal cocktail of growth factors used to maintain the embryoid bodies is responsible for triggering the cells' Wnt-responsive pathways. The researchers identified one specific factor, called bmp, that gets the ball rolling. Inhibiting this factor stops the spontaneous formation of the primitive streak in the embryoid bodies and gives the researcher more precise control over the cells' differentiation.

"Differentiation is a step-wise process," said Nusse. "To get to a particular endpoint, you need to know all the steps along the way. Our research indicates that embryoid bodies are a better-than-expected model of what happens in the embryo, and suggests how we may be able to manipulate those steps to our advantage to get pure populations of certain types of cells for research or therapy."

Additional Stanford researchers involved in the study include postdoctoral scholar Christophe Fuerer, PhD, research assistant Matt Fish and graduate student Elif Eroglu.

The research was supported by the Howard Hughes Medical Institute, the California Institute of Regenerative Medicine, the National Institutes of Health and the Swiss National Science Foundation.

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