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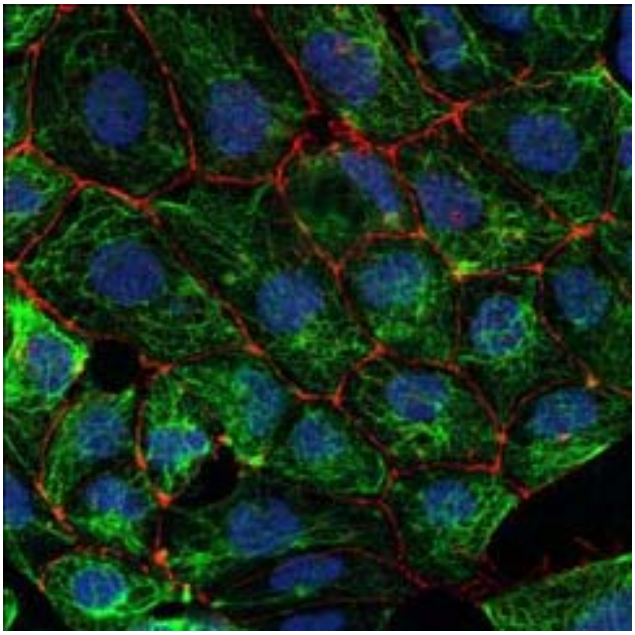
Fulfilling their bipotential: Techniques for monitoring and guiding differentiation of mesendodermal lineages from ES cells

December 1, 2005 – Embryonic stem cells are noted for their pluripotency – the ability to give rise to every type of cell in the body – but it has proven challenging to steer them to differentiate into specific lineages in culture. A detailed knowledge of the mechanisms that guide the embryo in vivo can provide valuable clues toward achieving that goal, but again, much remains to be clarified in the understanding of development as well.

At 3.5 days after fertilization, the mouse embryo is a hollow ball, called a blastocyst, made up of an outer layer of trophectodermal cells that go on to form placenta and an inner cell mass (ICM) of pluripotent cells, with a natural ability similar to that of ES cells in vitro to generate all of the body's cells. These ICM cells are initially unspecified, but through a process known as gastrulation they gradually differentiate into the tissues of the three germ layers: ectoderm, mesoderm and endoderm. The development of the endodermal layer is a complicated affair involving a pair of sub-lineages known as visceral endoderm, which contributes to extraembryonic tissues, and definitive endoderm, which give rise to embryonic endodermal cells such as gut, liver, pancreas and lung. The complexity doesn't end there however, for a subset of the definitive endoderm is reckoned to function as mesendoderm, the precursor material that will ultimately give rise to both mesoderm and endoderm as the embryo matures. This ability has been confirmed in a number of species, including the *C. elegans* roundworm and the African clawed frog, *Xenopus laevis*, but mesendodermal bipotentiality remains putative in mouse and other mammals.



Endodermal-derived epithelial cells differentiated from mouse ES cells;
Co-expression of E-cadherin (red), Foxa2 (blue), cytokeratin18 (green)

Now, in a series of experiments using mouse ES cells, the Laboratory of Stem Cell Biology (Shin-Ichi Nishikawa; Group Director) has demonstrated a means for inducing their differentiation both to visceral endoderm and, via an intermediary mesendodermal step, to definitive endoderm and mesoderm. This work now makes it

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possible for the first time to track these differentiation events in culture and to steer differentiation toward each of these lineages at high efficiency and to develop purified populations of cells sorted by their expression of surface antibodies. The studies have been described in a pair of reports published separately in the October issue of *Development* and the December issue of *Nature Biotechnology*.

Looking at patterns of gene expression in the region of the early embryo from which the endoderm derives, they identified *goosecoid* (*gsc*) as a factor likely to be involved in endodermal differentiation and surmised it might have utility as an endodermal marker. The group next knocked-in a version of the gene tagged with a green fluorescent protein and experimented with the cells in vitro until they had a method of inducing differentiation that yielded a population of cells nearly all of which glowed green (signalling that their expression of *gsc*.) Next they screened the *gsc*$^+$ colonies and determined that cells positive for goosecoid, e-cadherin and PDGFRα (gsc$^+$ECD$^+$$\alpha$R$^+$) possessed the twofold potency to differentiate into both mesoderm and endoderm characteristic of mesendoderm in other species. Even more intriguingly, this mesendodermal population could be subdivided into gsc$^+$ECD$^-$ αR$^+$ and gsc$^+$ECD$^+$$\alpha$R$^-$ complements, which the group found differentiated into mesoderm and definitive endoderm, respectively. Turning to cells in which the goosecoid gene was not fused to express fluorescent protein, they demonstrated that the ECD and αR did their job equally well in genetically unmodified cells.

Armed with this knowledge, the lab subsequently created a cell line in which the goosecoid locus bore the gene for gfp and huCD25 was introduced into the sox17 locus (sox17 is a known marker for extraembryonic visceral and definitive endoderm). This double knock-in enabled them to sort the definitive (gsc$^+$sox17$^+$) from the visceral (gsc$^-$sox17$^+$) fractions as the cells differentiated and, from there, to develop culture media best suited to nudging cells toward either of these lineages. Comparing gene expression in definitive and visceral endoderm, they identified seven surface proteins whose patterns of expression differed between populations. One of these molecules, CXCR4, is expressed specifically in definitive endoderm, meaning that by creating a monoclonal antibody against this protein, Nishikawa et al were able to track differentiation in unadulterated ES cells as well and to obtain pure populations of definitive endoderm by cell sorting.

The pluripotency that defines ES cells stands behind their allure as a source for populations of cells that might one day be used in cell replacement therapy. The research presented in these two recent articles first used genetically altered ES cells to study and identify surface markers characteristic of each lineage of interest. This allowed them next to follow the initial differentiative events of the mesendoderm in unmodified ES cells and, most importantly, to successfully purify the resulting populations a feat that may in the future make it possible to obtain large quantities of clinically useful cell types from endodermal organs such as pancreas and liver. Indeed, by both providing the first clear evidence of a mesodermal stage in mammalian gastrulation and at the same time opening a path toward healthcare strategies of great promise, these studies stand as a testament to the power of bipotentiality.