Identification of gene that promotes differentiation of pluripotential cells through analysis of classical mouse mutant

November 9 2012

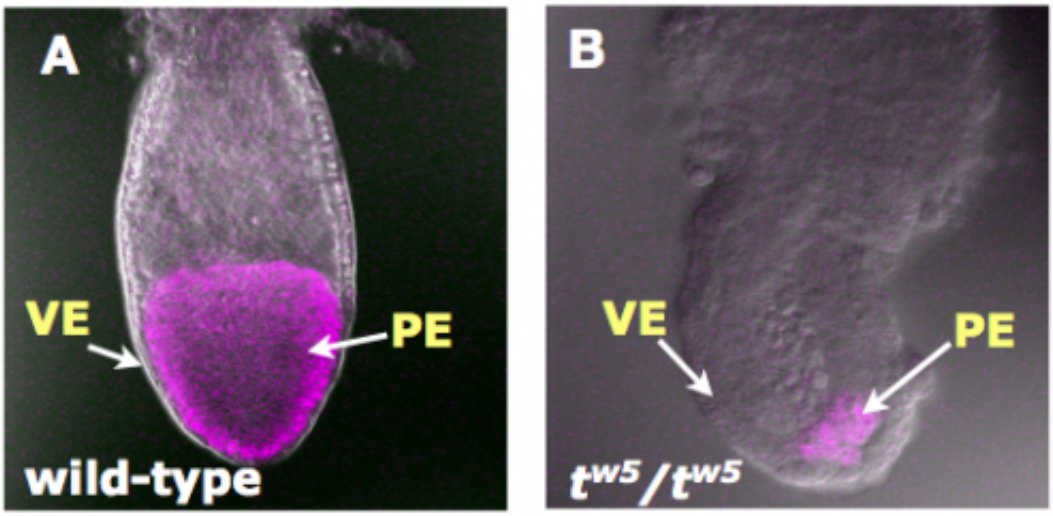

Figure 1. Developmental defects in the primitive ectoderm of tw5/tw5 embryos.

Researchers at RIKEN BioResource Center and their colleagues identified a gene required for growth and differentiation of pluripotential cells in the mouse embryos. The gene, *Vps52*, is a mouse homolog of yeast *VPS52* that is thought to be involved in the retrograde endocytic trafficking. The research group found that *Vps52* promotes differentiation of pluripotential cells including ES cells via cell-cell interactions, revealing hitherto unknown functions of *Vps52* in development of a multicellular organism. The findings, which appear in

the journal *Cell Reports*, should provide clues to the interrelations between endocytic machinery and developmental cell signaling in mammalian embryos, and also contribute to development of technologies that facilitates manipulation of pluripotent stem cells such as ES (embryonic stem) cells and iPS (induced pluripotent stem) cells.

During an early phase of mammalian development, developmental pluripotency is maintained by a particular cell lineage. After implantation, pluripotential cells are converted to primitive ectoderm through cell-cell interactions. This developmental transition is associated with significant changes in transcriptional and epigenetic networks. However, it is largely unknown which factors or genes are important for the postimplantation changes. Mutants displaying specific defects in the relevant processes provide an unbiased entry point for analyses. The t^{w5} mutation, occurring in the "*t* haplotype" shows highly specific defects in the primitive ectoderm of pregastrulating embryos, resulting in embryonic death at about 6.5 days after fertilization (embryonic day 6.5) (Figure 1). The *t* haplotype is a naturally occurring variant form of the mouse chromosome 17 that harbors large inversions that cause crossover suppression between *t* haplotype and wild-type (WT) chromosomes. As *t* haplotypes do not recombine with WT chromosomes, abundant genetic reagents and resources developed for analysis of WT chromosome cannot be applied. As a result, the recessive t^{w5} lethal mutation has not been identified molecularly to date, although the first report on the t^{w5} mutation was published in 1957. The researchers combined modern genome analysis technologies with BAC (bacterial artificial chromosome) transgenic rescue experiments to identify a candidate gene, *Vps52*, and confirmed its authenticity.

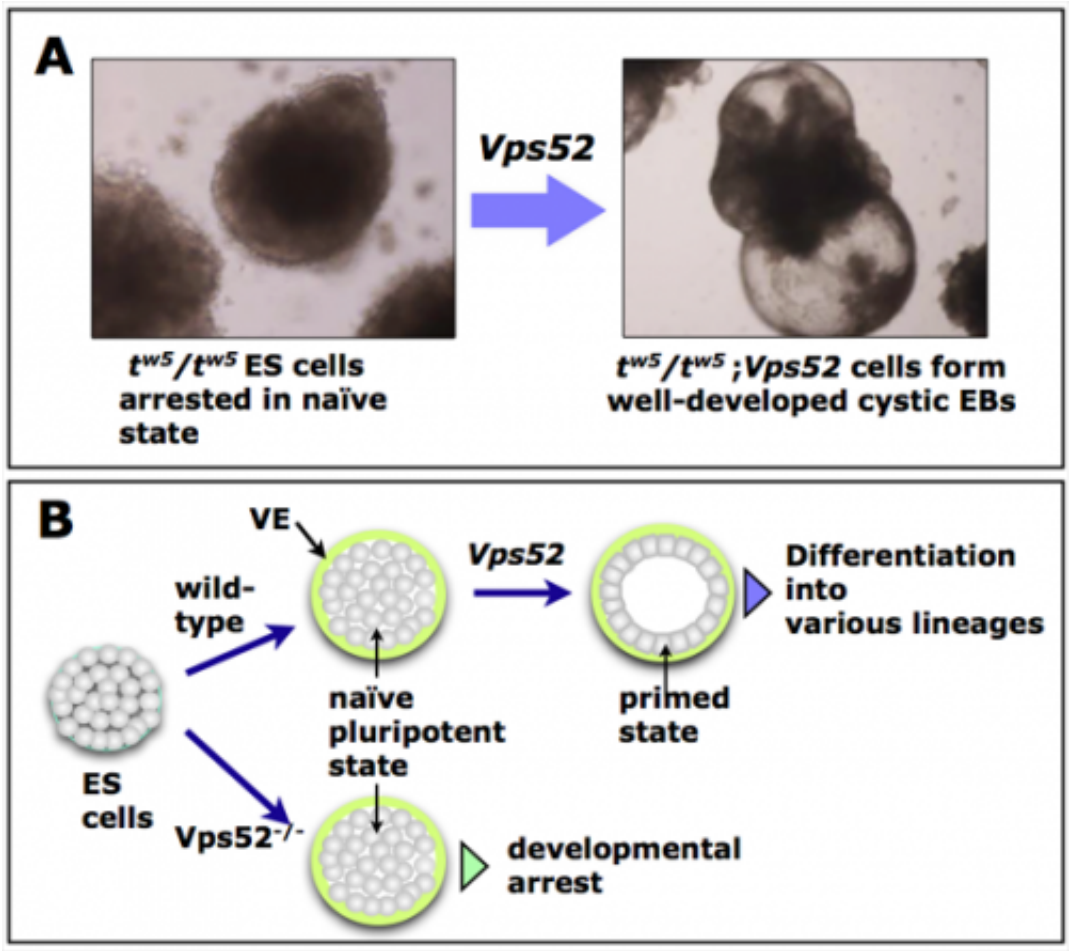

Figure 2. Vps52 expression promotes in vitro differentiation of ES cells. (A) tw5/tw5 ES cells are arrested as cell aggregates (left), whereas introduction of the Vps52 gene (tw5/tw5;Vps52) leads to efficient differentiation of cystic EBs (right). (B) Model for the function of Vps52 in the differentiation of pluripotent cells. VE cells (green) emerge from an aggregate of naïve pluripotent cells and form a layer of cells surround the pluripotent cells. Vps52 expression in the VE is required for transition from the naïve to the primed state.

Vps52 is the mouse homolog of yeast *VPS52* gene thought to be involved in the retrograde trafficking. The data strongly suggest that in mammals *Vps52* acts in extraembryonic tissues to support the growth and differentiation of primitive ectoderm, and is also required for embryonic vasculogenesis at a later stage of development.

Defects of t^{w5} null embryos are reconstituted in the embryoid body differentiation system of ES cells (Figure 2A). Upon induction of differentiation, t^{w5}/t^{w5} ES cells aggregate but cease the development, arrested in a "naïve" pluripotent state. Introduction of the *Vps52* transgene rescued the differentiation defect in the t^{w5}/t^{w5} ES cells, leading to formation of well-developed embryoid bodies. The developmental transition from a naïve to a primed state of pluripotent stem cells (or vice versa) (Figure 2B) is currently under intense investigation in the field of stem cell biology. *Vps52* is required for this transition. Thus it should be possible to facilitate this conversion process through the manipulation of *Vps52* activity.

More information: Sugimoto, M., Molecular identification of tw5:Vps52 promotes pluripotential cell differentiation through cell-cell interactions. *Cell Reports*, 2012, doi.10.1016/j.celrep.2012.10.004

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