Lsd1 – a gatekeeper for differentiation onset of embryonic mouse stem cells

Epigenetics is an emerging field of research that studies heritable changes in gene expression that are not caused by changes in the underlying DNA sequence. Prof. Dr. Roland Schüle, Director of Central Clinical Research at the Freiburg University Medical Centre, and his team are specifically focused on epigenetic modifiers that regulate the timely development of placental mouse tissue. Schüle and his team have discovered in mouse embryos that a specific enzyme induces stem cells to migrate and differentiate. The researchers' findings could be of interest for the fields of oncology and regenerative medicine.

Epigenetics encompasses all interactions between genes and their proteins that are not encoded in the DNA itself and that lead to changes in gene expression. The activity of gene segments and even entire chromosomes can be altered with epigenetic marks (e.g. DNA and histone methylation, etc.). Epigenetic modifications can silence DNA regions or make them physically accessible to the transcription machinery and hence enable their expression. While all cells of a developing mammalian embryo are identical up to the eight-cell stage, from then onwards, the cells undergo a cell-specific programme and differentiate into specialised types of cells with many different functions.

The functional identity of the cells depends on the activation or inactivation of specific genes. This can either be due to the highly specific biochemical modification of individual bases of the DNA sequence or to the biochemical modification of individual amino acids of the histones around which the DNA strand is wrapped. These epigenetic changes are preserved when cells divide. Epigenetics gives rise to major differences in the epigenetic modification distributions and hence to different cell types, including germline cells, stem cells, cancer cells and somatic cells.

Prof. Dr. Roland Schüle, Director of Central Clinical Research at the Freiburg University Medical Centre and member of the BIOSS Centre for Biological Signalling Studies at the University of Freiburg, is studying epigenetic mechanisms and is specifically focused on the methylation of histones. Gene silencing and expression is mostly controlled by proteins that mediate epigenetic changes through the acetylation of histones and by proteins that bind to methylated histones, but RNA molecules have also been shown to mediate gene silencing.

Reading, removing and interpreting

Prof. Dr. Roland Schüle studies the enzyme Lsd1 and its function in stem cells
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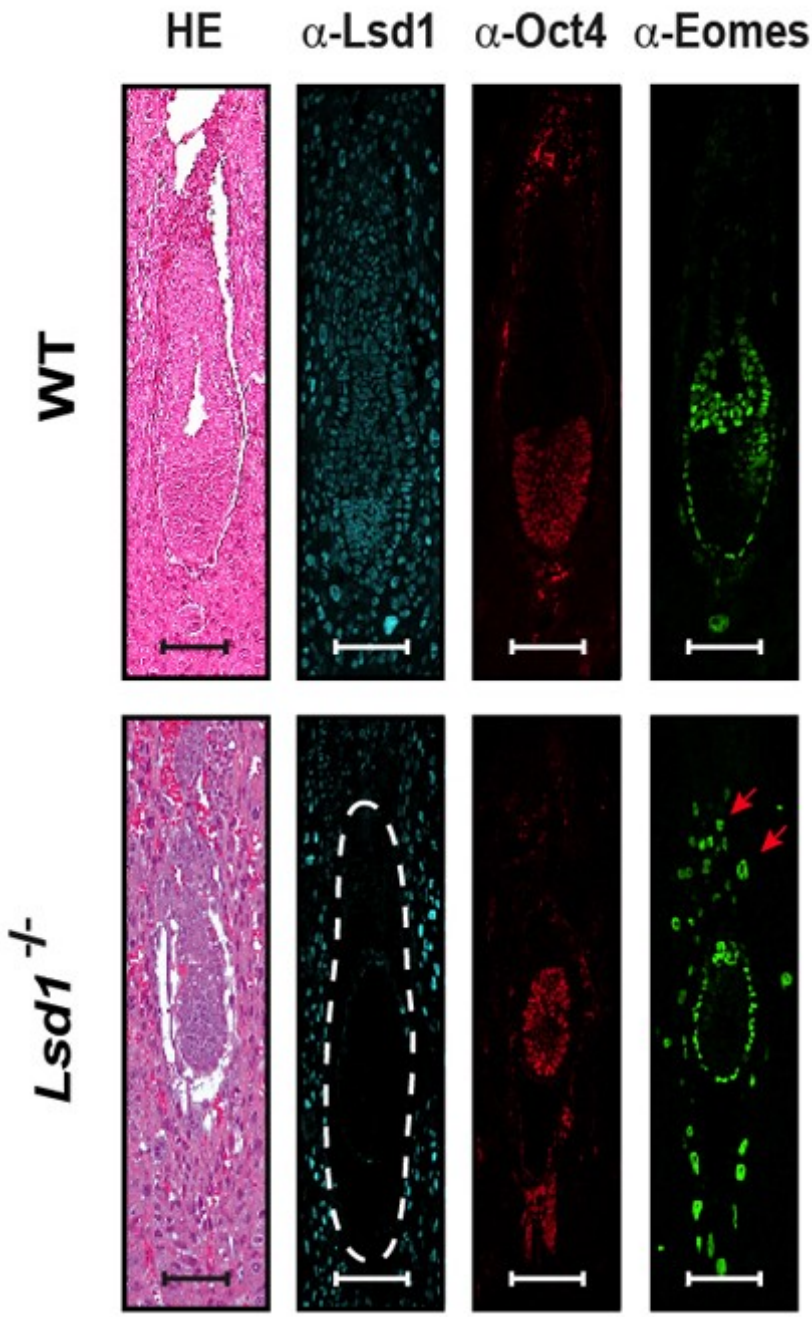
Schüle classifies epigenetic protein modifiers into three groups: writer proteins, which attach epigenetic tags such as methyl groups to the chromatin; reader proteins, which read or interpret the epigenetic ltags; and eraser proteins, which remove the epigenetic tags. The third group includes an enzyme in which Schüle is particularly interested. This enzyme, which is known as lysine-specific demethylase 1 (Lsd1), removes the methyl group from the amino acid lysine in histones.

Schüle's favourite enzyme brought him to stem cell research when he discovered that Lsd1 played a key role in mammalian embryonic development. It appeared to be a vital protein. The researchers were able to show that when they switched the protein off, the mouse embryos died in the womb within a couple of days. "Why does the mouse die? What is the molecular mechanism behind it?" asks Schüle and adds: "Our analyses showed that Lsd1 is a key regulator of stem cells." During embryogenesis, the blastocyst develops from the fertilized egg a few days after fertilization. The blastocyst consists of two layers, the inner cell mass (embryoblast) and the outer trophoblast where the embryonic placenta and the egg membranes form. In order for this to happen, the trophoblast stem cells must develop into particular cell types.

The tight regulation of cell propagation and differentiation of the trophoblast stem cells guarantees that a sufficient quantity of cells is available for the formation of placental tissue, while at the same

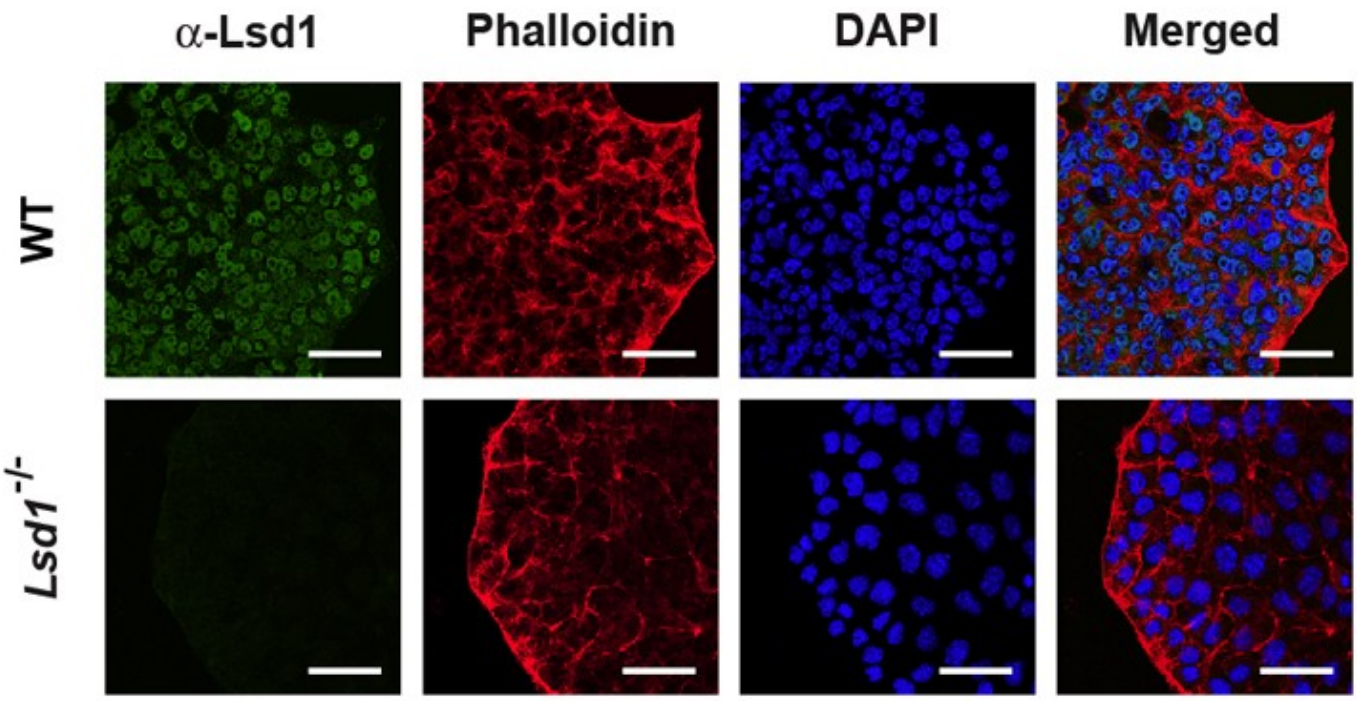


Stem cell markers (Oct4, Eomes) visualize the different distribution of trophectoderm cells in wild type (WT) and Lsd1-deficient mutants (Lsd1 -/-) of six-day-old mouse embryos. The trophoblast cells of the mutants are located outside the stem cell niche (red arrows; scale: 200 µm)

time maintaining a reservoir of stem cells (= stem cell niche). The researchers found that a specific signal is necessary in order to make the trophoblast stem cells migrate to the paternal placenta and establish an embryonic placenta required for the provision of oxygen and nutrients. In fact, the researchers found that Lsd1 was the central gatekeeper for the correct time of differentiation.

Loss of Lsd1 leads to stem cell mobility

During migration, the cells start to differentiate into giant trophoblast cells, so-called spongio- or syncytiotrophoblasts, that later form different regions of the placenta. On closer inspection, it becomes clear that a stem cell that loses Lsd1 gains the ability to migrate. This stem cell now lacks the information that tells it to remain a stem cell and stay in its original site until its time for migration has come. This is due to a second protein, the transcription factor Ovol2. Lsd1 suppresses



Lsd1 regulates the migration of trophoblast stem cells. The cytoskeleton of WT and Lsd1-deficient mutants is stained red and the nuclei blue (scale: 100 µm)
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the transcription factor Ovol2 in the as yet undifferentiated cells by removing the methyl group from the Ovol2 promoter, thereby preventing the cells from differentiating, migrating and invading the maternal placenta prematurely. Lack of suppression leads to the activation of Ovol2 at an earlier point in time than normal, and the cells start to migrate.

At the same time, the cells preferentially differentiate into giant trophoblast cells, rather than into the three different placental cell precursors. "Lsd1 regulates two functions, namely the ability to migrate and correct differentiation, which appear to have nothing in common, at least not at first sight," explains Schüle whose team is currently studying in detail how Lsd1 controls these two functions. The researchers are specifically focused on finding out whether this is due to the removal of methyl groups from the chromatin or more likely due to the lack of demethylation.

Cell differentiation and cancer

Although little is yet known about the role of Lsd1 in the adult organism, it is known that Lsd1 plays a key role in the formation of fat cells (adipogenesis) and also regulates the differentiation of neurons into olfactory receptors in the mouse brain. Switching off the gene in the pituitary gland impedes the growth of these mice. Schüle and other researchers have previously shown that Lsd1 plays a key role in the differentiation of stem cells.

In principle, it is therefore possible to specifically regulate Lsd1 and control the generation of specific cell types. This is promising for the field of regenerative medicine. Schüle's team is attempting to gain a detailed understanding of the role of Lsd1 by specifically looking at the dysregulation of the Lsd1 enzyme. "Our approach is focused on the physiology and pathology of the protein. We overexpress or turn off the Lsd1 gene in living mouse models in order to study the effect of its product," says Schüle. Schüle's team also works with knock-in mice with a specific mutation. This enables them to study whether the function observed depends on the presence of a multi-enzyme complex or on the Lsd1 protein alone. "We have created a mouse that only expresses enzymatically

inactive Lsd1," says the scientist. "This helps us to elucidate the role of the enzymatic function of Lsd1 in living mice."

The overexpression of Lsd1 leads to the development of prostate and lung tumours in old mice. Over the next few years, the researchers will focus on finding out why this happens. Small biotechnology companies have already shown an interest in the histone demethylase Lsd1 and are working on the development of Lsd1 inhibitors for the treatment of cancer.

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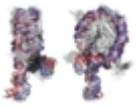

Epigenetics – heritable traits without changing the DNA sequence

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