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Inhibition of bromodomain affects stem cell differentiation

DNA methylation and histone modification are epigenetic mechanisms that affect gene transcription. Moreover, protein complexes can regulate gene expression by modifying chromatin structure and function. Dr. Thomas Günther and his team from the Center for Clinical Research at the Freiburg University Medical Center are studying the effect of the inhibitor PFI-3 on the BAF complex. This protein complex modifies chromatin structure and controls the maintenance and differentiation of stem cells. This might be interesting for regenerative medicine applications.

Four days after fertilisation, the mouse embryo starts to form a multilayered hollow sphere of cells called a blastocyst. The inner cell mass is formed by embryonic stem cells (ESC) which later become the complete mouse. The outer trophoblast stem cells (TSC) become the embryonic part of the placenta. These two types of stem cells differ in gene expression, which also changes over time. Stem cells can divide asymmetrically and form two non-identical daughter cells. One of these cells becomes a stem cell and the other differentiates into a special cell type. On the one hand, stem cells are characterised by their ability to give rise to an indefinite number of the same type of cells, and on the other, by their ability to differentiate into other cells. "While this definition of stem cells serves most goals, it is far too superficial to be used in the field of medicine," says Dr. Thomas Günther from the Center for Clinical Research at the Freiburg University Medical Center.

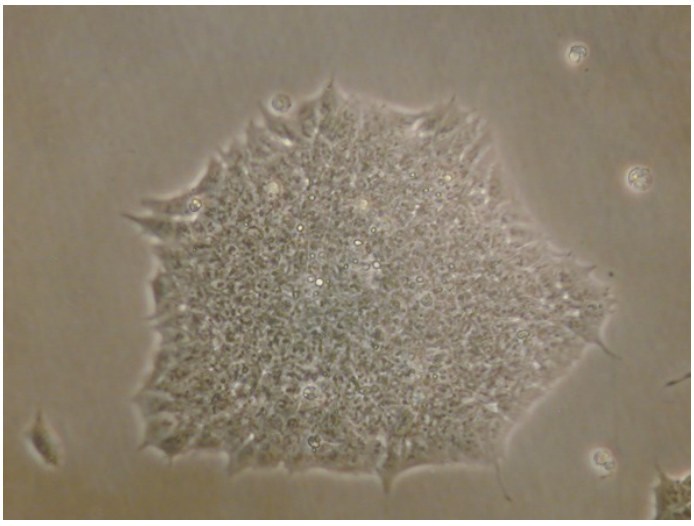
Bromodomain is of key importance

Dolly the sheep was created from adult somatic cells and presumably aged quickly because it had abnormalities in the epigenetic modifications of its DNA. Which epigenetic factors can be used to induce the generation of stem cells and subsequently make them become specific cells? Stem-cell development is controlled by an enzymatic machinery on the epigenetic level which modifies the packaging of DNA. This machinery is referred to as BAF complex (Brahma-associated factors), and it has the ability to alter the position of histones along the DNA strand. This leads to the exposure of the transcription binding domains in the chromatin, enabling transcription factors to access and bind to DNA. The remodelling of chromatin thus activates gene expression. BAF belongs to the family of chromatin remodelling complexes that perform different cell type-specific and context-dependent functions. Günther's research focuses on the BRG1 (Brahma-related gene 1) subunit of the BAF complex. BRG1 is a protein that uses energy from ATP to disrupt the chromatin structure. "Embryos that do not have BRG1 die at an early stage," says Günther. BRG1 contains a

bromodomain that selectively recognises and binds acetylated histone lysine residues. This domain is a key element in how post-translational modifications are read, and influences which genes are transcribed.

BAF maintains stem cell characteristics

What makes stem cells differentiate? Dr. Thomas Günther would like to find an answer to this question using the BAF complex.

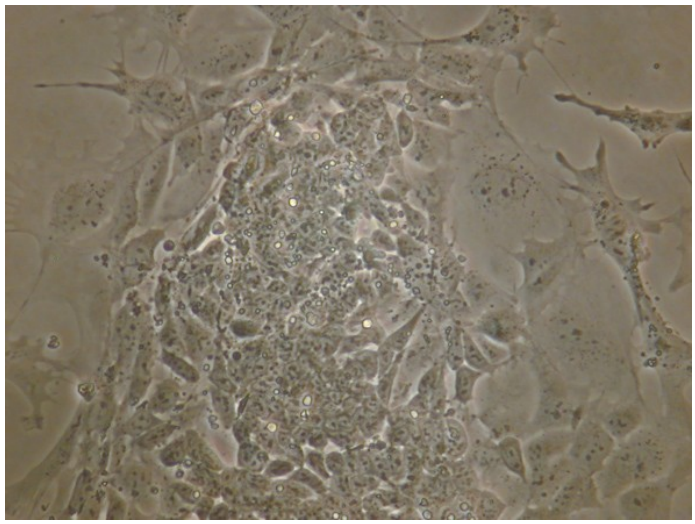


Embryonic stem cells that are kept in cell cultures grow in a characteristic way. They form clusters.

During stem cell development and differentiation, BAF is associated with regulators that activate transcription programmes that then trigger cell proliferation. "We know that the complex is required for ESC maintenance and also for proper lineage specification," says Günther. "Inactivating the complex triggers cell differentiation. ESC maintenance relies on the four Yamanaka factors Oct4, c-Myc, Sox2 and Klf4, whose expression is co-regulated by the BAF complex. Gene expression is downregulated when the complex does not bind to these factors, and the cells lose their stemness. In order to understand the function of BAF, Günther tried to identify inhibitors that prevented the BRG1 bromodomain from binding to the acetylated lysine residues. Using an acetylsalicylic acid derivative, Günther's colleagues from Oxford were able to identify a compound that fitted into the bromodomain of BRG1. It also bound in vitro. The compound used was an aspirin derivative and is called PFI-3. The questions Günther asked in an effort to elucidate BAF function were: What happens when PFI-3 binds? Does it have a biological effect? Günther's team were able to show that the bromodomain is crucial for the function of both embryonic and trophoblast stem cells because the gene transcription process, which controls cell differentiation, changed upon long-term exposure to PFI-3. The compound mimics the effect of a

BRG1 deletion by blocking a binding site. The fates of embryonic and trophoblast stem cells were studied using stem cell-specific molecular markers.

Stem cell differentiation with PFI-3



The inhibitor, an aspirin derivative (PFI), causes the cells to leave the cell collective and separate. These cells then lose their stem cell character.
© Dr. Thomas Günther, Freiburg University Medical Center

"If the effect of the inhibitor reflects the deletion of the BAF subunit, we expect expression of stem cell markers to be reduced too," summarises Günther. "We observed that the embryonic stem cells lost stem cell character." The cells' morphology reflects this loss. In cell culture systems, murine stem cells form small clusters. They lose their characteristics when exposed to PFI-3, they look different, leave the clusters and appear as individual cells. Further differentiation markers need to be analysed in order to find out what these cells are. "Different markers that are characteristic of different cell fates will be tested. For example, nestin, which is characteristic of neuronal precursor cells, or brachyury, which is required for posterior mesoderm formation in mice." The inhibitor therefore influences cell differentiation. The researchers found that more neuronal precursors and fewer mesodermal cells were formed in the presence of PFI-3. This is due to the fact that the BAF complex is not only able to control the expression of stem cell markers, but also the expression of differentiation markers. Whether it controls the expression of stem cell markers or differentiation markers depends on the context. PFI-3 also influences TSC, albeit on its own. "When the factor FGF4 is added to the stem cell culture, the cells remain stem cells, including in the presence of PFI-3," says Günther. "In the absence of FGF4, the cells start to differentiate, and do so more rapidly and strongly than when the inhibitor is present." The strength and speed of TSC differentiation rely on these factors, the maintenance of stemness does not. This effect has not previously been known."

Epigenetic drug target?

There are only a handful of drugs available that can modulate the expression of epigenetic target proteins. In principle, epigenetic mechanisms are excellent drug targets because epigenetics only affects proteins (including enzymes), but not the underlying DNA. In Germany, it is legally forbidden to re-isolate, generate and keep human embryonic stem cells in the laboratory. As stem cells can turn into any other human cell, they are an interesting target for regenerative medicine applications. Knowing how differentiated cells can be turned into stem cells, generate new stem cells and redifferentiate into specific cell types could in future be important for patients who require tissue replacements. The cells could be grown in cell culture systems and equipped with characteristics of specific organ tissues, thus speeding up the treatment of patients with trauma, bone fractures and genetic defects.

Article

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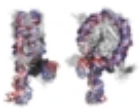

Epigenetics – heritable traits without changing the DNA sequence

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