Romanian Academy

Institute of Cellular Biology and Pathology "Nicolae Simionescu"

PhD THESIS

Epigenetic mechanisms involved in stem cell differentiation

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Keywords

Acetylation
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Differentiation
Neovascularization
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Thesis abstract

Importance of the study

Understanding the mechanisms which lead to differentiation of stem cells is the primary focus of numerous studies. Accessibility of DNA to transcription factors depends on chromatin structure and its degree of compaction. Recent analysis of epigenetic changes in human and murine stem cells have provided new data on the properties of pluripotency of stem cells and their differentiation capacity. These mechanisms lead to a hierarchy of transcription, are mediated by transcription factors and are designed to control gene expression without altering the DNA. Multipotent stem cell capacity decreases with time due to repression of certain genes that presents an epigenetic "signature". Active genes in the stem cells are silent gradually with the passing of their progenitors, and another subset of tissue - specific genes are activated. This progression is achieved by selective expression of transcription factors that recognize and interact with various epigenetic changes in the chromatin. As a result of these events, chromatin becomes accessible for transcription in certain regions, allowing the necessary spatial and temporal control for stem cell differentiation. For example, protein HP1 (heterochromatin protein 1) distribution changes from a dispersed localization in embryonic stem cells to build more concentrated in distinct loci during cell differentiation. Histone acetylation was seen as a phenomenon correlated with an open chromatin conformation that allowed the expression of different genes involved in differentiation. Currently it has been observed that in acetylated state, many genes are repressed and thus differentiation to a specific cell line is blocked, maintaining the pluripotent state. Handling histone deacetylase activity could be a useful tool to generate specific cell populations in order to use them in transplantation. Discovering patterns of acetylation ("acetylation signature") involved in the differentiation of stem cells to different cell types, opening new opportunities at the interface between chemistry and stem cell biology, and can provide valuable information to improve applications of stem cells in tissue engineering and regenerative medicine.

In this context, **the aim of the thesis** is to investigate the role of histone acetylation in the differentiation of endothelial progenitor cells by analyzing the expression of different genetic markers and transcription factors that regulate the activity of these genes. Also, these studies investigate the effect of endothelial progenitor cells acetylation on the process of *„in vitro”* neovascularization.

The thesis is divided into two main parts: Part I - Current state of knowledge and Part II - Original contributions.

The first part presents the current state of knowledge and is organized into three sections.

Subchapter I present notions about the characteristics and properties of stem cells. In this chapter is presented the cell model used in the thesis, represented by endothelial progenitor cells and their potential clinical applications.

Subchapter II makes an overview of the main epigenetic modifications. It describes the mechanisms of action of these modifications and the enzymes involved in their implementation. Also in this section describes the role of epigenetic changes in stem cell differentiation to various cell lines.

Subchapter III describes the mechanisms of histone acetylation and their role in stem cell differentiation. This chapter presents the main classes of histone acetyltransferase and histone deacetylases, histone acetylation mechanisms of action and influenceof acetylation in endothelial progenitor cell differentiation.

In Part II - "Original Contributions" are presented the results of experiments aimed to investigating the role of histone acetylation in the differentiation of endothelial progenitor cells and in neovascularization *in vitro*.

Chapter II.2 - " Materials and Methods " describes the main materials and techniques used in the experiments, including many molecular and cell biology techniques: Real -Time PCR, Western blot, flow cytometry, transmission electron microscopy and scanning electron microscopy, immunocytochemistry and immunohistochemistry, colorimetric and fluorimetric methods.

Chapter II.3 - " Results and discussion " presents the main original results obtained in the studies.

Results and discussion

Understanding the mechanisms which lead to the differentiation of stem cells is the primary focus of numerous studies. Recent analysis of epigenetic changes in human and murine stem cells have provided new data on the properties of pluripotecy of stem cells and their differentiation capacity. These mechanisms lead to a hierarchy of transcription, are mediated by transcription factors and are designed to control gene expression without altering the DNA. Histone acetylation was seen as a phenomenon correlated with an open chromatin conformation, allowing the expression of various genes involved in differentiation.

Currently it has been observed that in acetylated state, many genes are repressed and thus differentiation to a specific cell line is blocked, maintaining the pluripotent state.

In this thesis we have shown that umbilical cord blood contains a rich population of endothelial progenitor cells with an characteristic immunophenotype profile: CD31, CD34, CD133, CD144, CD146, VEGFR2. Molecular analysis of cells obtained from umbilical cord blood showed the presence of genes that are involved in cardiovascular morphogenesis, such as genes for transcription factors GATA2, GATA3, GATA4, and genes that are characteristic of endothelial cells: CD31, VE- cadherin, VEGFR1, VEGFR2, vWF, CXCR4, Tie -2. We have also shown that endothelial progenitor cells have a high capacity of proliferation and migration in comparison with other fetal stem cells types. These cells can uptake acetylated LDL, and *Ulex europaeus* lectin, can form vascular network, suggesting their potential in angiogenesis and vascular repair. This was confirmed using murine embryonic ventricular sections viable or subjected to ischemia, in which we showed that endothelial progenitor cells have the ability to integrate and form vascular networks. Our results indicated that endothelial progenitor cells forming the vascular structures only on viable murine embryonic ventricular sections, while in the ischemic sections only integrate, which demonstrates a direct cell-to cell communication.

The results showed that histone acetylation inhibit the differentiation of endothelial progenitor cells. Inhibitors of deacetylation (VPA, TSA, BuA) mentain chromatin in an acetylated state corresponding to a decondensate conformation. Histone deacetylases level was significantly decreased in the presence of these inhibitors and histone H3 acetylated level was increased and these changes are correlated with the expression of differentiation markers involved in endothelial progenitor cells commitment. The molecular biology and immunophenotyping data showes that HDAC inhibitors have inhibited the expression of vWF, VEGFR2 , eNOS , CD117 , CD133 , CD144 , CXCR4 and Tie- 2, while the expression of CD34 and CD45 remains unchanged showing that the histone deacetylases are involved in the differentiation of endothelial progenitor cells. VE-cadherin expression was significantly inhibited both at the mRNA and protein level. The mechanism underlying the expression of VE-cadherin may be explained by inability of HoxC6 transcription factor to interact with acetylated histones in order to activate the VE-cadherin promoter. Gene expression of CXCR4, Tie-2 and VEGFR2 significantly decreased after treatment with TSA, the mechanism involved is controlled by Hox transcription factors whose expression is modulated in the presence of these inhibitors. Our results have shown that in the acetylated state HoxD9 expression in endothelial progenitor cells is increased in both gene and protein level.

The process of neovascularization is a complex process involving a series of interconnected steps leading to the formation of new blood vessels when vascular lesions occur in adult bodies. In contrast to angiogenesis, which is the formation of new blood vessels from some pre-existing one, neovascularization takes place via endothelial progenitor cells. These cells are mobilized to migrate, proliferate and differentiate at the target site, under the action of cytokines and the microenvironment. Our results have shown that histone acetylation inhibits neovascularization *in vitro*, acting in the processes of proliferation, adherence, migration and differentiation of endothelial progenitor cells. Endothelial progenitor cells showed a significantly decreased level of telomerase activity in comparison with the control, suggesting a decrease in cell proliferation.

Cell motility assessed using the "*wound-healing assay*" and cell impedance measurement, showed that HDAC inhibitors decrease cell motility at 24 hours after stimulation. In contrast, chemotaxis of endothelial progenitor cells was increased after treatment with HDAC inhibitors, which can be explained by the maintenance of a circulating progenitor phenotype. In the presence of 3 mM VPA our data showed a significant stimulation of chemotaxis of endothelial progenitor cells to angiopoietin, VEGF and SDF. After proliferation, mobilization, migration and adherence of endothelial progenitor cells to the target situs, the last step consists in organizing their vascular network. We analysed also this step in terms of acetylation. Growing on Matrigel in the presence of HDAC inhibitors endothelial progenitor cells can not form vascular networks. To confirm these results we used other collagen-based matrix. The results indicated that endothelial progenitor cells isolated from umbilical cord blood were able to survive and adhere to the collagen matrix. Moreover, these cells emit extensions, interact with each other and with the matrix, forming in the network structure. In acetylated state, endothelial progenitor cells lose this ability, which demonstrates that acetylation inhibits neovascularization potential of endothelial cells *in vitro*.

Handling histone deacetylase activity could be a useful tool to generate specific cell populations for transplantation . Discovering patterns of acetylation ("acetylation signature") involved in the differentiation of stem cells to different cell types, opening new opportunities at the interface between chemistry and stem cell biology, and can provide valuable information to improve applications of stem cells in tissue engineering and regenerative medicine.

Number of figures in the first part -32

Number of figures in the original contribution (Part II) – 40

Bibliographical notes – 251

Papers published in international journal (ISI) – 4

Papers published in national journals (CNCSIS B +) – 1

Oral communications: 2

Abstracts of papers presented at international scientific meetings – 11

Abstracts of papers presented at national conferences – 25

Participation in research projects - 2 national, 1 international