Biochimie 139 (2017) 56–73

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Research paper

The effect of histone acetyl-transferase inhibitor trichostatin A on porcine mesenchymal stem cell transcriptome

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ARTICLE INFO

Article history:
Received 10 March 2017
Accepted 23 May 2017
Available online 25 May 2017

Keywords:
Mesenchymal stem cells
Pig
Transcriptome
Trichostatin A

ABSTRACT

The use of histone acetyl-transferase inhibitors such as trichostatin A (TSA) for epigenetic transformation of mesenchymal stem cells (MSCs), whose nuclei will be transferred into enucleated oocytes, is a novel approach in research involving somatic cell cloning of pigs and other mammalian species. Although the effectiveness of TSA in cloning applications was confirmed, processes and mechanisms underlying achieved effects are not yet fully understood, especially for pig MSCs. To contribute to this knowledge, in this study we performed a comprehensive transcriptome analysis using high-throughput sequencing of pig bone-marrow derived MSCs, both treated and untreated with TSA, and evaluated the effect of TSA administration on their transcription profile after 24 h of *in vitro* culture. The expression of selected positive and negative mesenchymal surface antigens was also evaluated in these cells by flow cytometry. Subsequently, the stability of induced expression changes was evaluated after another 55–72 h of culture without TSA. The results of this study showed that TSA does not affect the expression of the selected surface antigens related to MSC mesenchymal stemness origin, namely: CD90 (positive marker), CD31 and CD34 (negative markers) and has a wide stimulating effect on MSCs transcription, affecting genes across the whole genome with some minor signs of site-specific acting in regions on SSC2 and SSC6. TSA turned out to have a higher impact on already expressed genes with only minor abilities to induce expression of silenced genes. Genes with expression affected by TSA were related to a wide range of biological processes, however, we found some evidence for specific stimulation of genes associated with development, differentiation, neurogenesis or myogenesis. TSA also seemed to interfere with Wnt signaling pathways by upregulation of several engaged genes. The analysis of cell transcriptome after prolonged culture following the TSA removal, showed that the expression level of majority of genes affected by TSA is restored to the initial level. Nonetheless, the set of about six hundred genes responsible for e.g. adhesion, signal transduction and cell communication was altered even after 55–72 h of culture without TSA. TSA also enhanced expression of some of pluripotency marker genes (*FGF2, LIF, TERT*) but their expression was stabilized during further culture without TSA. The detailed analysis of factors connected with neuron-like differentiation allowed us to assume that TSA mostly stimulates neurogenic differentiation pathway in the pig MSCs possibly through interaction with Wnt-mediated signaling and thus triggers mechanisms conducive to epigenetic reprograming.

1. Introduction

Mesenchymal stem cells (MSCs) are attractive candidates for cell therapy purposes because of their potential for multilineage differentiation, biomolecular secretion and immunomodulatory properties [1,2]. Although MSCs are distributed in a number of different tissues such as bone marrow and adipose tissue [3,4] they represent a rare cell population, constituting approximately from 0.001% to 0.01% of nucleated cells in bone marrow [4]. Therefore *ex vivo* expansion remains an indispensable procedure to obtain sufficient amounts of MSCs for cell therapies and tissue

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http://dx.doi.org/10.1016/j.biochi.2017.05.015

engineering. The ability of MSCs to differentiate into various lineages and their immunomodulatory properties are their major therapeutic advantages, but *in vitro* culture of stabilized MSC lines to obtain high quality cells is still a challenge. Mechanisms underlying MSC differentiation into a particular lineage have been under active investigation and recent studies suggest that epigenetic regulation is a vital mechanism of MSC differentiation [5].

DNA methylation in CpG sites and histone modifications are major mechanisms responsible for epigenetic regulation of gene expression during development and differentiation in mammals [6,7]. One of histone modifications is histone acetylation which is important for nucleosome assembly and chromatin dynamics. Histone acetylation promotes open chromatin states by modulating interactions between nucleosomes and releasing histone tails from linker DNA [7]. Chromatin regions that are enriched in lysine acetylation catalyzed by Histone Acetyl-transferase (HATs) are in most cases actively transcribed, whereas regions that are bound by Histone Deacetylases (HDACs) bear deacetylated lysines and stay inactive [8]. HATs and HDACs may act either in a site-specific manner, when they are bound to sequence-specific DNA binding activators or repressors, or in a broad manner acting across large genomic regions. Genome-wide detection of HATs and HDACs in higher eukaryotic organisms has revealed a highly complex mechanism in which active genes were bound by both enzyme types, whereas inactive genes were bound by HDACs [9].

HDAC inhibitors (HDACis) are natural or synthetic molecules that can suppress the activities of HDACs. One of HDACis is trichostatin A (TSA) which is an organic compound that serves as an antifungal antibiotic and selectively inhibits class I and II (but not III) mammalian HDACs [10]. The effect of TSA on MSC differentiation and proliferation seems to be dosage-dependent [11]. It was found that human MSCs treated with low dosage of TSA in *ex vivo* cultures showed increased expression of pluripotent genes such as *Oct4, Sox2, Nanaog, Rex-1* and *TERT*. Administration of low concentrations of TSA also significantly suppressed morphological changes in MSCs occurring during culture expansion, increased their proliferation while retaining their cell contact growth inhibition property and multipotent differentiation ability [11]. Other study also showed that HDACis stimulate self-renewal and suppress differentiation and at low doses (10 nM), revert mouse embryoid bodies towards the undifferentiated state [12]. Nevertheless, at higher concentrations (>150 nM), TSA was shown to be able to induce cell differentiation processes or even direct cells to an apoptotic pathway [13].

The effect of TSA administration seems to be also cell-type dependent. Some studies showed that HDACis (in accordance with their effects on the differentiation of cancer cells) are able to promote the differentiation of human embryonic stem (ES) cells. Several studies reported that treatment, even with a low dosage of trichostatin A, stimulates morphological and gene expression changes reminiscent of differentiation even in the presence of pluripotency transcriptional regulator − LIF [14,15]. The inhibition of HDAC activity was correlated with the acceleration of the early differentiation steps of ES cells without being sufficient for commitment to a specific lineage. Additionally, a genome-wide analysis performed with cDNA microarrays on human ES cells revealed the presence of two gene groups that are targeted by TSA after 6–12 h of treatment: the first one consisted of genes related to pluripotency which were suppressed by TSA (*Sall4, Nanog, Klf4, Oct4,* and *Sox2*) and the second one included genes required for lineage-specific differentiation, which were upregulated by TSA [15]. This study also showed that a large part of TSA-mediated transcriptome changes were completely reversible after several hours of culture without TSA.

Despite numerous studies performed in mice and humans, up to now, little is known about gene expression and their regulation in the porcine MSCs. Virtually nothing is known about the impact of epigenetic modulators such as TSA on the porcine MSC transcriptome. Although it was shown that at low concentrations TSA stabilizes the expression of pluripotent genes, it is largely undefined how this HDAC inhibitor influences whole transcriptome in the pig MSCs and what is the mechanism behind the observed suppression of the morphological changes of cells during cell culture or their increased proliferation abilities [11]. It is also largely unknown how stable are the epigenetic modifications introduced by TSA administration. In this research we focused on the pig which is a species widely used as a model for human diseases due to its similarities in anatomy, physiology, and immune response as well as a fairly long life span (reviewed in Prather et al. [16]). The utility of pigs as a model for human diseases has been recently largely enhanced by genetic engineering aiming for modifications of xenoantigens triggering hyperacute transplant rejection. Well characterized porcine cell lines with proven multipotency have also great potential in transgenesis and biomedical research (mainly in the field of regenerative medicine and tissue engineering) e.g. by using them for somatic cloning of transgenic pigs for xenotransplantation purposes. Results of our previous works showed the higher efficiency of TSA-modulated bone marrow derived pig MSCs compared to somatic cells used as nuclei donors for somatic cell nuclei transfer [17,18]. Therefore, in the present research we attempt to identify the mechanisms responsible for the greater usefulness of epigenetically modulated MSCs for cloning purposes. The bone marrow-derived MSCs were used as they are still the most frequently investigated cell type and often designated as the gold standard [19].

To address all the above issues, we performed a comprehensive transcriptome analysis using RNA-Seq method of pig bone-marrow derived MSCs, both treated and untreated with TSA, and evaluated the effect of TSA addition on their transcription profile after 24 h of culture. Additionally, the stability of induced expression changes was evaluated after another 55–72 h of culture without TSA. As an additional aspect of the study we investigated whether TSA-induced modifications affect the expression of the selected surface antigens related to mesenchymal stemness origin in MSCs.

2. Material and methods

2.1. Animals and ethic statements

Five outbred Polish Large White (PLW) male pigs, weighing 15–20 kg each, maintained under conventional conditions at the Institute's Experimental Station in Rudawa were used for the study. All animal procedures were approved by the Local Animal Care Ethics Committee No. II in Kraków at the Institute of Pharmacology of the Polish Academy of Sciences, permission number 1256/2015 in accordance with EU regulations.

2.2. Isolation and in vitro culture of porcine mesenchymal stem cells

The pig mesenchymal stem cells (MSCs) were isolated as described in Opiela et al. [20]. Briefly, the bone marrow was aspirated from the iliac crests of the animals under general anaesthesia. Mononuclear cells were collected by gradient centrifugation at 400 × g for 20 min over a layer of Ficoll-Paque Plus (Stem Cell Technologies; SCT, Canada). Single-cell suspensions were seeded at a concentration of 1×10^5 cells/cm^2 per 75-cm^2 culture flask (Corning, USA) containing 17 ml medium comprised of low-glucose Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, Germany) supplemented with 10% foetal bovine serum (FBS; Sigma-