Journal of Stem Cells Research, Reviews & Reports

Austin Publishing Group

Open Access Full Text Article

Letter to the Editor

Circadian Expression of DEC1, SMAD3 and SNAIL in Human Mesenchymal Stem Cells

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Received: July 29, 2014; **Accepted:** July 30, 2014; **Published:** July 31, 2014

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Mesenchymal Stem Cells (MSCs) derived from human and animals can be cultured *in vitro* and play important roles in the development of bone, cartilage, and adipocytes, bone repair, and skeletal regeneration [1,2]. It has been reported that MSCs are also associated with tumor microenvironment, indicating that MSCs have multiple functions *in vivo* [3]. However, it is not well understood whether MSCs have a circadian rhythm. We have reported the circadian expression of clock genes (CLOCK, BMAL, PER, CRY, DEC) in the Suprachiasmatic Nucleus (SCN), peripheral tissues of rat and mouse, and differentiated human and animal cells. In addition, we have recently demonstrated that clock genes and transcription factors SMAD3 and SNAIL show circadian expression in human MSCs [4]. This is a new finding on clock genes because the significance of clock genes in MSCs is not clear. Generally, the mechanism of circadian rhythms depends on the molecular negative feedback system by clock genes. CLOCK and BMAL1 heterodimer (CLOCK/BMAL1) binds to an E-box in the promoter of PER, CRY, or DEC, to promote their transcription and translation. Some PER, CRY, and DEC proteins are degraded by phosphorylation, ubiquitination, or SUMO lylation, and the remained proteins of a dimer of PER and CRY or DECs suppress CLOCK/BMAL1 transactivation [5-7]. This negative feedback system plays important roles in circadian regulation in normal and tumor cells [8,9]. We think that the molecular negative feedback system may be applied to MSCs because most of the clock genes show circadian expression in MSCs. Because MSCs do not completely initialize DNA and undifferentiation, we sought to examine the circadian rhythm of clock genes in MSCs by serum shock, which fully induced the expression of clock genes, SMAD3, and SNAIL. We examined whether CLOCK/BMAL1 affected SMAD3 transactivation because we found that the SMAD3 gene has E-boxes in its promoter. As a result, the SMAD3 promoter activity was induced by CLOCK/BMAL1 co-transfection, whereas CLOCK/BMAL1 had little effect on the SNAIL transactivation. These results suggest that serum shock is suitable for observing the circadian rhythm in MSCs and that the circadian rhythm of SMAD3 may depend on CLOCK/BMAL1 transactivation through E-boxes. However, itis still unknown how the circadian expression of SNAIL is regulated. Further studies are needed to clarify the molecular mechanism of the circadian expression of SNAIL.

SMAD3 is a receptor-regulated SMAD in transforming growth factor-beta (TGF-β) signaling, which regulates cell growth, proliferation, apoptosis, and differentiation [10,11]. TGF-β induces differentiation of MSCs to vascular smooth muscle cells via nuclear translocation of SMAD3 [12]. Inhibition of SMAD2/3 signaling promotes enrichment of human embryonic-stem cell-derived MSCs [13]. These phenomena by SMAD3 may be driven by a circadian rhythmal though there are few reports on the circadian rhythm. It has been reported that SMAD3 binds to the DEC1 promoter and DEC1 in turn shows circadian expression in SCN, peripheral tissues, and hMSCs [4,5,14]. DEC1 induces differentiation of MSCs to chondrocytes, whereas it suppresses adipogenic differentiation [15].These results suggest that differentiation to chondrocytes and suppression of adipogenic differentiation in MSCs driven by DEC1 may occur under the circadian rhythm and be regulated by SMAD3. The research on circadian rhythms of both SMAD3 and DEC1 in MSCs may be interesting in future experiments. It has been reported that TGF-β activates DEC1 and SNAIL via the phosphorylation of SMAD3 [16]. The transcription factor SNAIL regulates epithelial markers E-cadherin and claudins and mesenchymal markers N-cadherin, vimentin, and α-SMA, inducing Epithelial-Mesenchymal Transition (EMT) [16,17]. A recent paper reported that SNAIL showed the circadian expression in breast cancer cells, inducing EMT [18]. Furthermore, SNAIL prevents the differentiation of MSCs to osteoblasts and adipocytes [19]. Thus, DEC1, SMAD3, and SNAIL show the circadian expression in normal cells, tumor cells, and MSCs to regulate the differentiation, cell growth, and EMT under the circadian rhythms.

Interestingly, there is no circadian expression of clock genes in Induced Pluripotent Stem (iPS) and Embryonic Stem (ES) cells, which are not differentiated [20,21]. They showed that circadian oscillation was observed by day 15 after differentiation in mouse. These results suggest that the circadian rhythm plays an important role in aging. It has been reported that disturbance of circadian rhythms may cause diseases, such as Alzheimer's disease, diabetes, metabolic syndrome, and cancers [22-26]. It seems likely that circadian rhythms of older adults may cause more disorders than those of younger people. The number of MSCs is decreased with aging, which may delay wound healing and immune response. Therefore, it would be important to keep circadian rhythms of MSCs to prevent diseases. Detailed mechanisms why circadian rhythms are required after differentiation are still unclear. It is not well understood the precise function of DEC, SMAD3, and SNAIL in MSCs, iPS, and ES cells. There are many articles on the function of these molecules in differentiated cells without considering circadian rhythms, whereas few articles address the circadian rhythm of DEC [27]. It needs to consider the circadian expression as well as differentiation and proliferation of stem cells by

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DEC, SMAD3, and SNAIL. In the future, it should be clarified how these molecules play important roles in circadian rhythms in stem cells.

Cancer stem cells are associated with resistance to anti-tumor drug and malignancy [28]. Also, DEC1, DEC2, PER1, CLOCK, and BMAL1 are associated with drug resistance and malignancy [29,30]. However, it is not still understood whether cancer stem cells show a circadian rhythm and a significant expression of clock genes. It would be interesting if cancer stem cells show circadian expression and the drug resistance is associated with clock genes.

DEC1, SMAD3, and SNAIL play important roles in differentiated cells, but the roles in iPS and ES cells are still unknown. A previous report showed that over expression of Oct3/4, c-Myc, Klf4, and Sox2 induced iPS cells from mouse fibroblasts [31]. DEC1 directly regulates c-Myc expression, cell proliferation, and apoptosis in normal and tumor cells [32,33]. It would be possible that DEC1 may play important roles in iPS cells, involving c-Myc expression. Future studies should clarify how these molecules are associated with the roles in ES and iPS cells.

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J Stem Cells Res, Rev & Rep - Volume 1 Issue 1 - 2014
ISSN : 2381-9073 | www.austinpublishinggroup.com

Citation: Sato F, Muragaki Y. Circadian Expression of DEC1, SMAD3 and SNAIL in Human Mesenchymal Stem Cells. J Stem Cells Res, Rev & Rep. 2014;1(1): 1001.