OSTOGENIC POTENTIAL OF HUMAN ADIPOSE-DERIVED ASC52TELO CELL LINE

KAROLINA TRUCHAN, KAROLINA LELEK, ANNA MARIA OSYCZKA*

DEPARTMENT OF BIOLOGY AND CELL IMAGING,
INSTITUTE OF ZOOLOGY AND BIOMEDICAL RESEARCH,
JAGIELLONIAN UNIVERSITY, POLAND
*E-MAIL: ANNA.OSYCZKA@UJ.EDU.PL

[ENGINEERING OF BIOMATERIALS 153 (2019) 82]

Introduction

Bone marrow stromal cells (BMSC) and cells from the stromal vascular fraction of adipose tissue (ASC) are now extensively studied for several tissue engineering approaches. In 2001 Zuk *et al.* described adipose-derived mesenchymal cells which could differentiate into multiple lineages, including osteoblasts [1]. Harvesting such cells from patients is less invasive compared to BMSC and results in less morbidity and higher amounts of osteoprogenitors in initial isolates and for further expansion in culture. Treatments of bone defects may include ASC instead of BMSC although ASC are reported to display lower osteogenic potential vs. BMSC [2]. Standard osteogenic inducers in culture include ascorbic acid phosphate, dexamethasone, β-glycerophosphate [3]. Bone morphogenetic proteins are known as potent osteogenic growth factors *in vivo* and *in vitro* and BMP-2 and -7 are already used in clinics [4]. In this study we have investigated the osteogenic potential of hTERT immortalized adipose derived mesenchymal stem cell line (ASC52telo, ATCC SCRC-400) that may serve as a useful model to study osteogenesis in adipose-derived MSC. In addition, given that several studies suggest that silencing BMP natural inhibitor Noggin may improve the process of differentiating stem cells towards osteoblast [5], we have investigated the role of Noggin in the osteogenesis of ASC52telo and other MSC of different than adipose tissue origin. This was important in the view of the latest finding that showed that Noggin binds to BMP receptors and it can induce osteogenic effects in osteoblastic cells [6].

Materials and Methods

ASC52telo cells were expanded in culture medium composed of 89% MEM Alpha, 10% FBS Q and 1% Penicilin-Streptomycin. Osteogenesis was induced with different combinations of dexamethasone (Dex), ascorbic acid phosphate (Asc), β-glycerophosphate (BGP) and bone morphogenetic protein-2 (BMP-2). Osteogenesis was evaluated by biochemical assay of cellular ALP activity at 7-day culture, the collagen and mineral staining at 14-day culture. All results were presented as means and SD. Statistical significant differences were assessed by one-way analysis of variance followed by Tukey's tests for multiple comparisons and $p<0.05$ was considered significant.

Results and Discussion

The highest increase in ALP activity in ASC52telo cells was observed after stimulation of cells with Asc and Dex. Notably, ALP activity levels after cell stimulation with Asc+Dex with and without 100 ng/ml BMP-2 were not significantly different. Dex seemed to play a key role in stimulation of ALP activity in ASC52telo cells (FIG. 1). Collagen synthesis was stimulated by Asc and Asc+BGP (FIG. 2). These results confirmed the crucial role of ASC in the synthesis of collagen by osteoblastic cells [3].

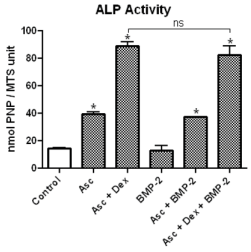

Fig 1. Alkaline phosphate activity after 7-d culture of ASC52telo cells with osteoinductive factors and BMP-2.

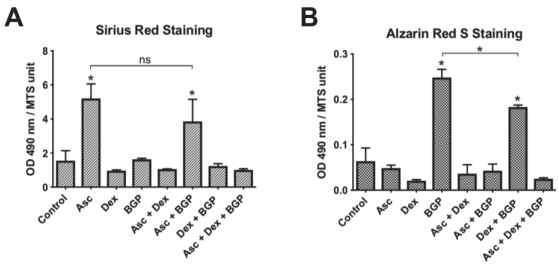

Fig 2. A - Collagen levels after 14-d culture of ASC52telo cells with osteoinductive factors. **B** - Mineralization levels after 14-d culture of ASC52telo cells with osteoinductive factors. *p<0,05, ns p>0,05.

On the other hand BGP was necessary for extracellular matrix mineralization, but it was effective alone or with Dex, but not together with Asc (FIG. 2). Interestingly enough, we have also found that Noggin increased ALP activity of BMSC cells treated with Asc+Dex and ongoing studies are aimed at examination of Noggin potential to induce osteogenesis in ASC52telo and other adult humans MSC.

Conclusions

Based on current results ASC52telo cell line shows good osteogenic potential in culture when stimulated with standard osteogenic inducers, i.e Asc, Dex and BGP, but these cells seem poorly responsive to rhBMP-2 similar to human BMSC [7]. Further studies will focus on in depth analyses of osteogenic markers in ASC52telo cell line and other than rhBMP-2 potential osteogenic growth factors.

Acknowledgments

This work was supported by Grant No. UMO-2016/21/B/NZ5/00217 (AMO).

References

[1] Zuk, P. A. *et al.*, (2001) Tissue Engineering, 7(2), 211–228.
[2] Noe ̈l D. *et al.*, (2008) Exp Cell Res 314:1575–1584.
[3] Langenbach, F., & Handschel, J., (2013) Stem Cell Research & Therapy, 4(5), 117.
[4] Zhang, X. *et al.*, (2013) Tissue engineering. Part B, Reviews, 20(1), 84-92.
[5] Fan, J. *et al.,* (2013) PLoS ONE, 8(8), e72474.
[6] Hashimi, SM. (2019) J Cell Biochem. 2019; 120: 13237-13242.
[7] Osyczka, A. M., & Leboy, P. S., (2005) Endocrinology, 146(8), 3428–3437.