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Dexamethasone treatment during the expansion phase sustains stemness of mesenchymal stem cells from human bone marrow

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Rationale: For most applications in tissue engineering and cell therapy, the use of adult stem cells is advocated because of their ability to self-renew and to differentiate into different cell types. When grown under appropriate conditions, human bone marrow-derived mesenchymal stem cells (hBMSCs) can form various tissues. Dexamethasone (Dex) has been reported to stimulate osteogenic potential of hBMSC, while maintaining or increasing cell duplication rate during expansion. In this study, we have investigated the effect of Dex treatment (10^{-8} M) during the proliferation phase on hBMSC multipotency.

Methods: hBMSCs were isolated from bone marrow aspiration by adhesion selection, and expanded in alpha-MEM proliferation medium with or without 10^{-8}M Dex. Expanded cells from early passage (P2-3) or late passage (P6) were identified by a range of surface markers by flow cytometric analysis and further cultured in osteo-, adipo- and chondrocyte differentiation medium. Osteo-, adipo- and chondrocyte formation were detected under a light microscopy and quantitatively measured by calcium deposition, oil-red-o releasing and gycosaminoglycan (GAG) assay respectively. Furthermore, micro-array was performed to compare the gene expression profiles of hBMSCs when they were treated with or without Dex.

Results: Expanded hBMSCs positive (>70%) for mesenchymal and/or adhesion markers (CD105/CD29/CD44/CD166/CD90) and negative (<5%) for hematopoietic markers (CD34/CD45/CD14). Dex did not change cell surface marker expression. hBMSCs gave rise to specialized cell lineages upon culture in osteo-, adipo- and chondrocyte-genesis medium. Upon treatment with Dex during the expansion phase hBMSC showed higher potential to become osteo- or adipocyte. Interestingly, Dex prevented loss of proliferative potential of hBMSC upon sequential passaging. It also prevented loss of the typical hBMSC cell surface phenotype. Accordingly, Dex improved maintenance of multipotent stem cell capacity of late passage hBMSCs as demonstrated by osteo- and adipogenic potential. In concert with these observations, gene expression analysis of Dex treated hBMSC showed that Dex could negatively regulate transcription of genes correlated with apoptosis and differentiation, while genes implicated in cell proliferation were positively regulated by Dex.

Conclusions: Dex improves the quality of hBMSCs since delay of senescence, maintenance of differentiation potential are observed when hBMSCs are expanded in the presence of Dex.

***Keywords*:** dexamethasone, mesenchymal stem cell, expansion, senescence, differentiation, multipotency

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