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Changes of gelatinases activity in human amniotic membrane-derived mesenchymal stem cells cultured in a hepatogenetic medium

Jiyoung Kim[1], Sooyeon Park[1], Minji Kook[1], Yoonhee Lee[1], Hyunmi Kang[1], Byung-Rok Do[2] Haekwon Kim[1]

[1]*Department of Biotechnology, College of Natural Sciences, Seoul Women's University,* [2]*HURIM Bio Cell*

Introduction

Gelatinases are members of the matrix metalloproteinase (MMP) family responsible for the degradation of extracellular matrix and base-membrane components during remodeling of most tissues. Two forms of gelatinases have been identified namely a 72-kDa gelatinase A and a 92-kDa gelatinase B referred to as MMP-2 and MMP-9 respectively. Both gelatinases have a broad spectrum of substrate specification such as type IV collagen, laminin and fibronectin as well as gelatin. Basic fibroblast growth factor (bFGF), the prototypic member of a large family of heparin-binding polypeptides and a potent angiogenesis inducer modulates vascular endothelial cell proliferation, migration and proteinase production. bFGF also is known as a potent inducer in hepatogenesis using zymography and immunoblotting methods. The present study was conducted to investigate gelatinases in human amniotic membrane-derived mesenchymal stem cells (HAM) and to determine whether there are any changes in gelatinase profiles when the cells are cultured in hepatogenic medium.

Materials and methods

Placenta was obtained during caesarean section of the volunteers with informed consent. HAM were isolated from amniotic membrane using collagenase type A. HAM were cultured in hepatogenic medium for 3

weeks and the conditioned media were obtained at day 7, 14 and 21. Activity and protein expression of gelatinases were determined by zymography and immunoblottng.

Results

The zymographic pattern of gelatinolytic activity of the HAM did not undergo a change during passages. When the HAM were cultured in a fibronectin (FN) or collagen (COL) - coated dishes in a hepatogenic medium, there was no significant difference of the gelatinase pattern between before and after culture. However, when bFGF was added to the culture, a dramatic increase of 62kDa and 59kDa gelatinases was observed. Interestingly, when ITS instead of FN or COL was present, HAM-conditioned medium also showed a similar increase of both gelatinases. Immunoblotting analysis demonstrated that both 62kDa and 59kDa gelatinases were the active form of MMP-2 resulting from the turnover of MMP-2 proform.

Conclusions

When HAM were cultured hepatogenic medium for 3 weeks, addition of bFGF to the culture medium induces a significant increases of active MMP-2 form. Further study will be necessary to determine the relationship between bFGF and active MMP-2 during hepatogenesis of HAM.