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Cumulus cells conditioned medium facilitates germ cell development from human embryonic stem cells

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Background: Human embryonic stem cells (hESCs) can differentiate to germ cells as confirmed using gene expression assessments. Cumulus cells are physically closest cells to the developing oocyte and they have positive effects on oocytes maturation by secreting some factors that have a crucial role in the process of oogenesis. Thus, in vitro differentiation of embryonic stem cells may also be affected of cumulus-secreted factors.

Objective: The aim of this study is assessment the effect of the cumulus cells conditioned medium (CCCM) on differentiation of hESCs to female germ cells.

Materials and Methods: Embryoid bodies (EBs) from Yazd4-hESCs were formed and cultured for 14 days into 4 different conditions: 1) spontaneously

differentiation in EB medium (SD-EB), 2) treated with 40% CCCM (CCCM-EB), 3) spontaneously differentiation in 40% DMEM+20% FBS (SD-DM), and 4) treated with 40% CCCM in DMEM+20% FBS (CCCM-DM). Expression of pluripotency and germ cells genes were examined in EBs from each group by RT-qPCR at the time of days 0, 4, 7 and 14. In addition, immunofluorescent (IF) staining was done for pluripotency and germ cells markers.

Results: RT-qPCR data revealed that CCCM-EB and CCCM-DM had a significant increase in differentiation of female germ cell from hESCs than SD-EB and SD-DM. On the other hand, comparison between basal media revealed that EB medium is a better medium than DMEM+20% FBS for female germ cell development from hESCs. Localization of the germ cells within the cultures was detected using IF for TRA-2-49, SSEA1 and VASA antibodies in all groups.

Conclusion: Cumulus cells conditioned medium supports female germ cell development from hESCs assessed by gene expression profile. Also, EB medium as basal medium has better impact on differentiation induction.

Key words: Cumulus cells, Conditioned medium, In vitro gametogenesis, Human embryonic stem cells, Female germ cells.

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