Abstract

Introduction:

The somites are spherical aggregation of mesodermal cells which are located on either side of neural tube. They differentiate into ribs, vertebrae and muscles. The notochord, as an axial mesoderm that is beneath the neural tube has a major role in *in vitro* somitic cell survival and differentiation. This study was aimed to investigate *in vitro* antiapoptotic role of chicken notochord upon co-culturing with somite.

Methods: After isolation of notochord and somites from chick embryo (stages 7-12), the notochord was encapsulated in alginate beads. Then, the somites and notochord, as a 2:1 ratio (2 somites:1 notochord) were co-cultured for 2, 4, 6 and 10 days. The somites were also cultured with notochord-derived condition medium (CM). In the control group, the somites were remained untreated. All groups were cultured in DMEM/F12 + 10%FBs medium. Finally, survival and proliferation of somites, expression of apoptotic factors and caspase-2 enzyme activity in somitic cells were analyzed by using MTT, RT-PCR and spectrophotometry methods, respectively.

Results: Percentages of viable somitic cells at day 2, normalized with these cells at day 0, for somite with notochord (S+N), somite with notochord-derived CM (S+NCM) and somite alone were 59%, 51.3% and 64.1%, respectively. After 4 days co-culturing, 74.4%, 28.2% and 21.3% of somitic cells showed to be alive in above mentioned groups, respectively while 89.7 %, 53.8 % and 35.9% of cells respectively survived at day 6. RT-PCR analysis showed caspase-2 was not expressed in S+N while Bcl-2 was up-regulated more than the other groups. The somitic cells in S+N expressed high Pax1 and low MyoD. Spectrophotometry also revealed that Caspase-2 enzyme acitivities in S+N and Somite alone at day 6 were 1.5 and 1.2.

Conclusion: The notochord improves *in vitro* somitic cell survival and reduces its apoptosis. It also triggers somitic cells to diffentiate into sclerotomal cells.

Key words: Co-culture, Notochord, Somite, Apoptosis, Chick embryo.