Title: EXPERIMENTAL STUDY ON CARDIOMYOCYTES IN VIVO DIFFERENTIATED FORM MESENCHYMAL STEM CELLS

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OBJECTIVE To investigate the ability to differentiate in vivo into cardiomyocytes form Mesenchymal stem cell (MSCs).

METHODS MSCs were isolated from bilateral thighbones and tibias of Wistar rats, purified by adhesive-screening method expanded in culture in vitro and labeled with DAPI, then injected into the myocardium of the rat recipients with acute myocardial infarction model which were created by legation of left anterior ascending artery. At intervals myocardial specimens around injection site were obtained, sectioned and stained with hematoxylin and eosin and electron microscopy for studying morphological changes of implanted MSCs. myosin heavy chain (MHC) and cardiac-specific antigen Cx43 were detected by immunohistochemistry, cardiac early-developmental gene NKx2.5、GATA-4 were detected through RT-PCR.

RESULTS We found almost all of MSCs were labeled with DAPI. viable cells labeled with DAPI were identified in host myocardium at all time after implantation. Implanted MSCs showed the growth potential in myocardial environments. MSCs reorganized themselves from a disorder pattern around injection site to an order structure along the long axis of normal myofibers. Furthermore, labeled MSCs were in parallel with the native fibers and integrated fully with the native fibers. The intercalated discs were detected at 4th week after implantation. Implanted MSCs demonstrated myogenic differentiation with the expression of MHC at 2nd week and cardiomyocytes phenotypes with the expression of Cx43 at 4th week, meanwhile, cardiac early-developmental gene NKx2.5、GATA-4 expression and its quantities changes with interval time point were detected using RT-PCR. they display NKx2.5、GATA-4 expressed at 1day after MSCs implantation,their expression quantity were up to peak at 2 week and from then on diseased.

CONCLUSION In response to myocardial microenvironments implanted MSCs can differentiated into cardiomyocytes, which were confirmed in morphology, histology and cellular biology.