Derivation and isolation of mouse mesenchymal stem cells from induced pluripotent stem cells

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Introduction: Transplantation of adult donors'–derived mesenchymal stem cells (MSCs) is able to improve cardiac function in experimental acute myocardial infarction models. However, the poor survival rate of adult MSCs in ischaemic environment has limited its therapeutic efficiency. Previous investigations have indicated that MSCs derived from early embryonic stage such as embryonic stem cells or induced pluripotent stem cells (iPSCs) have greater potential of proliferation and differentiation. Nevertheless, up to date, therapeutic capacity of mouse MSCs derived from iPSCs remains elusive. This ongoing study therefore aimed to derive and isolate mouse MSCs from iPSCs for attenuation of tissue ischaemia. We hypothesise that transplantation of mouse-induced pluripotent stem cell–derived mesenchymal stem cells (iPSC-MSCs) will achieve a better therapeutic efficiency by enhanced retention or survival rate compared to adult bone marrow–derived mesenchymal stem cells.

Methods: We utilised a three stepwise method to derive and isolate mesenchymal-like stem cells from mouse iPSCs. Firstly, feeder cells and leukaemia inhibitory factor (LIF) were removed to induce iPSCs into spontaneous differentiation. The subsequent step is enrichment of MSCs by conditioned medium with basic fibroblast growth factor (FGF2) and epidermal growth factor (EGF) supplements. Lastly, isolation of enriched MSCs was analysed by fluorescence-activated cell sorting (FACS) to isolate subpopulation CD90+/CD133-. The single cell–derived MSC-liked colonies were expanded by limiting dilution. These cells were subjected to surface markers profiling and multipotent differentiation studies, namely adipogenesis, osteogenesis and chondrogenesis.

Results: The iPSC-derived cells were morphologically similar to adult bone marrow–derived MSCs. These cells were negative for CD34, anti Oct4, anti TRA-1-60 and CD133, while being positive for mesenchymal markers, CD44, CD73 and CD90. These cells were further induced into adipocytes, osteocytes and chondrocytes under differentiation-conditioned medium.

Conclusion: Derivation of mouse mesenchymal stem cells from mouse-induced pluripotent stem cells was successful. These cells hold potential therapeutic properties for ischaemic disease regeneration in near future.

Enhanced therapeutic efficacy of transarterial chemoembolisation treatment in hepatocelluar carcinoma (HCC) by mTOR inhibitor RAD001: implication for a novel treatment regimen in HCC

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Introduction: Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide. Transarterial chemoembolisation (TACE) is commonly used for the treatment of locally advanced HCC by dual actions of chemotherapy and ischaemic hypoxia. In this study, the therapeutic efficacy of RAD001, a novel mTOR inhibitor and its potential combinatory use with TACE for treatment of HCC using orthotropic HCC models was investigated.

Methods: Therapeutic efficacy of RAD001 and TACE was investigated in vivo and in vitro. For in-vivo studies, equal portions of established xenografts were orthotopically implanted into mice to generate HCC tumours. RAD001 was administered orally or by intraportal vein injection, hepatic artery ligation was performed to mimic transarterial chemoembolisation treatment, and chemotherapeutic effect was achieved by intraportal injection of cisplatin. For in-vitro studies, MHCC97L cells were treated with cisplatin and RAD001 under normoxic and hypoxic conditions to investigate their biological effects on HCC cells, including cell proliferation and regulation of different targets in mTOR pathway.

Results: Significant inhibition of tumour growth was observed when MHCC97L was treated with RAD001 and cisplatin in orthotopic mouse models. Combination of hepatic artery ligation combined with RAD001 treated through portal vein led to >90% tumour shrinkage when compared with the non-treated HCC controls. In addition, synergetic inhibitory effect was observed when RAD001 combined with cisplatin treated through TACE in orthotropic HCC model. In-vitro studies also showed inhibition of cell proliferation by 52% and 40% in normoxic and hypoxic condition, respectively, when treated with 10 nm RAD001 alone. Cell proliferation was further inhibited when treated in combination with 2 mg/mL of cisplatin by 56% under normoxia and 36% by hypoxia. Western blot analysis revealed inhibition of mTOR, p70S6K(thr389) and PRAS40 phosphorylation by RAD001, and also downregulation of HIF-1α via the mTOR pathway under hypoxic conditions.

Conclusion: RAD001 used in combination with TACE could effectively inhibit tumour growth via the mTOR pathway and also enhances the cisplatin-induced toxicity towards HCC, providing the basis for use of RAD001 in combination with TACE + cisplatin as an effective regimen for treatment of HCC.