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大鼠骨髓基质干细胞分化为胰岛样细胞

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Islet-like cells differentiated from rat marrow mesenchymal stem cells

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Abstract

AIM: To explore the possibility of differentiating functional islet-like cells from marrow mesenchymal stem cells.

METHODS: Rat marrow mesenchymal stem cells (MSC) were isolated from Wistar rats and cultured. Passaged MSCs were induced to differentiate into islet-like cells under following condition: LN group, pre-induced with L-DMEM including 10 mmoL/LNicotinamide +1 mmoL/L β-Mercaptoethanol +20 mL/L fetal bovine serum for 24 h,then induced with serum free H-DMEM solution including 10 mmoL/L nicotinamide +1 mmoL/L β-mercaptoethanol for 10 h. HN group, pre-induced with L-DMEM including 20 mmoL/L Nicotinamide +20 mL/L fetal bovine serum for 24 h, then induced with serum free H-DMEM solution including 10 mmoL/L nicotinamide for 10 h. Differentiated cells were observed under inverse microscope, insulin and nestin expressed in differentiated cells were detected with immunocytochemistry. Diabetes model was induced with streptozotocin (STZ) injection, and 3 diabetes rats were subcutaneously injected with 1×10^7 islet cells differentiated from MSC respectively, and blood glucose level was tested 1 week after cell injection.

RESULTS: Islet –like clustered cells were observed under both LN and HN groups, abundant insulin expression was detected in islet-like cells, and nestin was detected in pre-differentiated cells. Glucose level in STZ-diabetic rats could be effectively controlled by islet cells differentiated from MSC.

CONCLUSION: Islet-like functional cells can be differentiated from marrow mesenchymal stem cells, which may be a new procedure for clinical treatment of diabetes.

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摘要

目的: 探讨体外自体骨髓基质干细胞(MSC)定向诱导分化为胰岛样细胞的可能性, 为进一步诱导分化胰岛细胞治疗糖尿病奠定实验和理论基础.

方法: 对Wistar大鼠骨髓基质干细胞(MSC)进行体外培养, 通过不同的条件诱导MSC向胰岛样细胞分化: LN组, 10 mmoL/L尼克酰胺+1 mmoL/L β-巯基乙醇的L-DMEM(含20 mL/L FBS)预先诱导24 h, 10 mmoL/L尼克酰胺+1mmol/L β-巯基乙醇的无血清H-DMEM诱导10 h. HN组, 含20 mmoL/L尼克酰胺的L-DMEM(20 mL/L FBS)预先诱导24 h, 20 mmoL/L尼克酰胺的无血清H-DMEM诱导10 h. 诱导细胞形态通过倒置显微镜观察, 并通过免疫细胞化学检测分化细胞表达Insulin和Nestin. 向3只Streptozotocin(STZ)诱导糖尿病大鼠皮下种植MSC分化胰岛细胞1×10^7, 1 wk后观察大鼠血糖水平变化.

结果: LN、HN组细胞均可见部分骨髓基质干细胞分化、增生, 形成胰岛样细胞团, 部分细胞向神经元样细胞分化, 对照组细胞未见明显分化. 免疫细胞化学显示Insulin在诱导细胞呈显著表达, Nestin在分化前细胞显著表达. 皮下种植MSC分化胰岛细胞可有效降低STZ糖尿病大鼠的血糖水平.

结论: 体外诱导骨髓基质干细胞定向分化为胰岛样细胞存在可行性, 本实验将可能为临床糖尿病干细胞治疗提供新的途径.

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0 引言

骨髓基质干细胞(marrow mesenchymal stem cell, MSC)存在定向分化潜能, 已有报道MSC可以分化为脂肪细胞、软骨细胞、肌细胞等中胚层细胞和神经细胞等外

胚层细胞以及肝细胞等内胚层细胞[1-2]. 但对 MSC 是否可以在体外直接诱导分化为胰岛细胞国内外文献都未见报道. 我们发现，在体外培养大鼠 MSC，通过不同条件培养基对 MSC 进行定向诱导分化，可以稳定诱导部分 MSC 形成功能性胰岛样细胞团，在体实验初步证明 MSC 分化胰岛细胞可有效降低 STZ 糖尿病大鼠的血糖水平. 现将结果报告如下.

1 材料和方法

1.1 材料 Wistar大鼠购自同济医学院实验动物中心. L-DMEM、H-DMEM、胎牛血清(fetal bovine serum，FBS)等购自 GIBCO 公司，β-巯基乙醇、尼克酰胺、B27购自Sigma公司; Nestin、Insulin mAB购自Santa Cruz公司.

1.2 方法 无菌条件下取 4-8 wk Wistar 大鼠胫骨和股骨，用含 20 mL/L FBS 的 L-DMEM 冲出骨髓，充分吹打成单细胞悬液，200 目筛网过滤，调整细胞密度，按 1 × 10^9/L 密度接种，37 ℃、502 mL/L CO_2 的细胞培养箱培养 48 h 后更换培养液，弃去未贴壁细胞，此后每 3-4 d 换液 1 次. 约 10 d 待 MSC 接近长满瓶底时以 252 mL/L 胰蛋白酶消化、吹打成单细胞悬液，1 000 r/min 离心 10 min，弃上清，用含 20 mL/L FBS 的 L-DMEM 悬浮沉淀，按 1∶3 比例传代. 取传至 2-3 代的 MSC 细胞按 5 × 10^8/L 密度接种于置有盖玻片的 24 孔板内制备细胞爬片，待细胞长至 70-80% 融合时，按下列几种方法诱导 MSC 分化: LN 组，10 mmol/L 尼克酰胺 + 1 mmoL/L β-巯基乙醇的 L-DMEM(含 20 mL/L FBS)预先诱导 24 h，更换培养液，PBS 洗涤 3 次，再加入含 10 mmol/L 尼克酰胺 + 1 mmoL/L β-巯基乙醇的无血清 H-DMEM 诱导 10 h. HN 组，含 20 mmoL/L 尼克酰胺的 L-DMEM (20 mL/L FBS)预先诱导 24 h，更换培养液，PBS 洗涤 3 次，再加入含 20 mmoL/L 尼克酰胺的无血清 H-DMEM 诱导 10 h. 同时设立以下对照组: (1)不加任何诱导剂，(2)10 mmoL/L 尼克酰胺，(3)加 1 mmoL/L β-巯基乙醇. 不同时期诱导细胞在倒置显微镜下观察形态变化，免疫细胞化学检测细胞表达 insulin 和 Nestin 的表达水平.

1.3 分化细胞在体控制血糖效应的初步观察 取200 g左右健康大鼠经尾静脉注射 STZ 50 mg/kg 制备糖尿病模型.1 wk 后以 8 只血糖水平高于 24 mmoL/L 大鼠为实验对象，3 只大鼠各皮下注射诱导分化细胞 1×10^7，3 只大鼠各皮下注射未诱导分化细胞 1×10^7，2 只大鼠为接受皮下注射为空白对照. 注射细胞1 wk后测定各只大鼠血糖水平.

2 结果

2.1 MSC 的分离培养分化 将骨髓细胞悬液接种在培养瓶里，48 h 内可见大量贴壁细胞，此时换液除去未贴壁细胞，3-4 d 后可见数个细胞克隆，细胞为纺锤形，成网状或栏栅状排列，形态均一. 10-15 d 后 MSC 接近融合，传 2-3 代后 MSC 纯度达 99% 以上. 通过 LN、HN 诱导骨髓基质干细胞分化 30 min-2 h，光镜下可见部分细胞转分化为胰岛样细胞(图 1)，倒置显微镜下细胞呈圆形团状排列，细胞内含有丰富的内分泌颗粒，和 ESC 定向分化形成的胰岛样细胞相似. 并可见部分细胞分化为神经元样细胞. 而不同对照条件下培养的MSC细胞仍呈梭形，无上述胰岛样细胞形成.

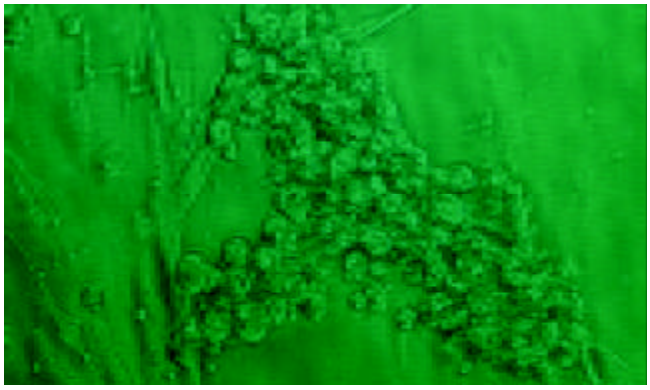

图 1 MSC 诱导分化为胰岛样细胞(× 40).

2.2 检测 Insulin 、Nestin 等的表达 在诱导分化为胰岛样细胞的胞质中可以见到大量的insulin阳性表达产物，而在未分化的梭形细胞未见到insulin表达(图2). 我们同时发现，nestin 在分化前梭形细胞呈阳性表达，尤其在向神经元样分化细胞中表达明显(图 3).

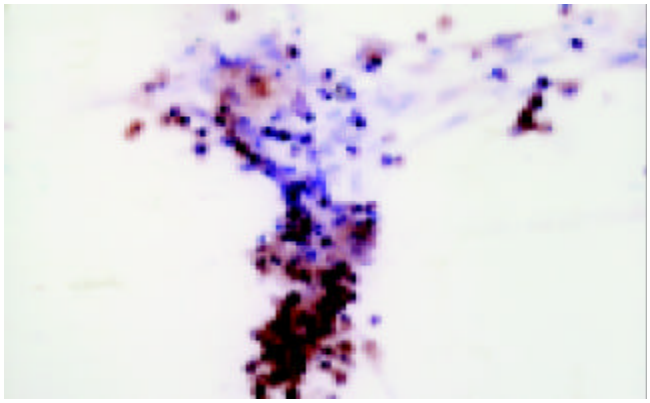

图 2 Insulin 在诱导分化细胞表达(× 10).

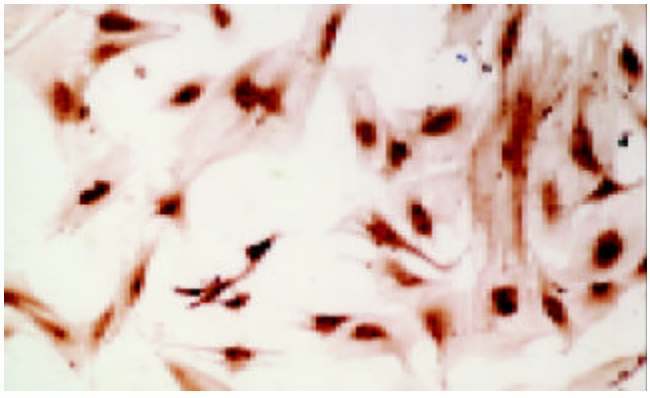

图 3 Nestin 在分化为胰岛样细胞前的 MSC 表达(× 40).

2.3 皮下种植MSC分化胰岛细胞对糖尿病大鼠血糖水平控制的初步观察见表 1.

表 1 MSC 分化胰岛细胞在体控制糖尿病大鼠血糖水平

不同注射组	注射前血糖水平(mmoL/L)	注射 1 wk 后血糖水平(mmoL/L)
分化细胞 1	>33.3	25.4
分化细胞 2	>33.3	21.4
分化细胞 3	25.3	19.7
未分化细胞 1	>33.3	>33.3
未分化细胞 2	28.9	29.7
阴性对照鼠	>33.3	>33.3

由于实验组动物数量较少且实验持续时间较短，我们未进行统计学处理. 尽管缺乏统计学依据，但从表 1 可以推测，和 MSC 相比，诱导分化形成的胰岛样细胞可有效降低糖尿病大鼠血糖水平.

3 讨论

糖尿病是世界范围的常见病，胰岛细胞移植是最有价值的治疗方法之一，但供体来源缺乏、胰岛细胞体外以及移植活力难以维持和移植排斥等阻碍了胰岛细胞移植的应用[3-7]. 胰腺导管上皮内干细胞[8-14]、胚胎干细胞(ESC)[15-20]和其他多种来源的干细胞[21-22]可分化为功能性胰岛细胞，但存在供体细胞来源不足或移植排斥等缺陷. 近来发现骨髓基质干细胞(MSC)可以诱导分化为脂肪细胞、成骨细胞、肌细胞、内皮细胞、神经细胞、肝细胞等多胚层细胞[1-2, 23-26]. 诱导MSC分化为功能性细胞理论上可以解决器官移植的主要障碍: (1)自体MSC定向分化后移植(Auto-graft)可以避免移植排斥; (2)骨髓干细胞来源丰富且具有巨大的增生效能[27]，可能解决供体来源不足问题，为临床提供足够的功能细胞或组织.

骨髓基质干细胞不仅可分化为中胚层细胞如脂肪细胞，也可以分化为肝细胞等内胚层细胞; 肝细胞和胰岛细胞来源于同一祖细胞[21]，肝细胞中的干细胞可以转分化为胰岛细胞[22]，都表明诱导骨髓干细胞分化为胰岛细胞理论上存在可行性. 在体实验也证明分化骨髓细胞可以在受体糖尿病动物胰岛中分化为胰岛样细胞，并有效调控血糖水平[28]. 因而，通过分离患者自体骨髓干细胞并诱导分化，将可能提供足够的功能性胰岛细胞，而不会发生移植排斥，具有重要的科研价值和临床应用前景. 但在体外直接诱导MSC分化为功能性胰岛样细胞尚未见报道. 我们通过分离培养大鼠 MSC，并采取适当条件成功诱导 MSC 向胰岛样细胞分化.

我们通过不同条件诱导 MSC 体外定向分化，首次发现MSC可以分化为胰岛样细胞团. 形态学和初步的功能鉴定结果可确定所诱导细胞为胰岛样细胞: (1)ESC 诱导的胰岛样细胞在形态上和本组结果相似[16]; (2)MSC 可向中胚层分化，本组诱导细胞形态上属于内胚层细胞，说明MSC 已被成功诱导跨胚层分化，内胚层细胞在适宜条件下可向肝细胞、胰岛细胞进一步分化，而尼克酰胺是较特异的胰岛细胞分化剂，因而本组细胞为胰岛样细胞; (3)本组中部分细胞向神经元样细胞分化，细胞分化过程中胰岛样细胞可能来源于神经元样细胞[8-9, 12, 16]，进一步支持本组细胞属于胰岛样细胞. 免疫组织化学显示 insulin 在诱导分化细胞呈特异性表达，从蛋白水平证实本组诱导细胞为功能性胰岛样细胞. 有报道 Nestin 阳性细胞是 ESC 等干细胞向胰岛样细胞分化过程中胰岛样细胞的前体细胞[12, 16]. 为进一步确定 MSC 诱导分化为胰岛样细胞的可能性，我们对胰岛细胞 Nestin 的表达进行检测，发现 Nestin 在胰岛样细胞分化前的细胞呈特异表达，尤其在部分向神经元样细胞分化的细胞中显著表达，进一步证明 MSC 向胰岛样细胞诱导的可行性. 有报道干细胞可通过细胞融合或黏附相应蛋白质而获得其他细胞表型[29-31]，本组中分化细胞性质还需要 RT-PCR 检察 insulin 基因转录、测定细胞分泌 insulin 等的进一步验证. 尽管缺乏统计学资料，我们的在体实验初步表明 MSC 分化胰岛细胞可有效降低糖尿病大鼠的血糖水平.

本文首次报道体外骨髓基质干细胞(MSC)可以诱导分化为胰岛样细胞. 尽管需要对分化细胞进行进一步的功能学鉴定、MSC 分化细胞的免疫学特性，以及明确分化细胞对糖尿病的治疗效应，我们的形态学和初步功能表明这些细胞为功能性胰岛样细胞. 本研究结果为通过 MSC 诱导胰岛样细胞、以及通过大规模制备分化的功能胰岛细胞治疗糖尿病提供了新的途径.

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