

移植医学专栏

小分子RNA干扰组织因子在新生猪胰岛细胞中的表达

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摘要:

目的:利用小分子RNA(siRNA)在基因水平对新生猪胰岛细胞进行修饰,抑制细胞组织因子的表达。 **方法:**设计5对siRNA转染新生猪胰岛细胞,用real-time PCR方法筛选组织因子基因沉默效果最好的siRNA或组合,同时流式细胞仪检测siRNA 转染对细胞活性的影响,real-time PCR和流式细胞仪分别检测细胞组织因子的基因及蛋白沉默水平。 **结果:**根据real-time PCR结果筛选出组织因子基因沉默效果最好的3对siRNA组合,转染新生猪胰岛细胞后,real-time PCR及流式细胞仪检测新生猪胰岛细胞组织因子的基因沉默效率达60%,蛋白表达水平降低约50%,同时流式细胞仪检测结果提示siRNA转染对新生猪胰岛细胞的活性没有明显的影响。 **结论:**3对siRNA组合在体外特异性抑制新生猪胰岛细胞组织因子的表达。

关键词: siRNA 组织因子 新生猪胰岛细胞

siRNA-mediated tissue factor knockdown in porcine neonatal islet cell clusters in vitro

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Abstract:

Objective To determine the genetic modification on neonatal porcine islet cell clusters (NICC) by small interfering RNA (siRNA)-mediated tissue factor (TF) knockdown in vitro. **Methods** Porcine NICC were transfected with 5 pairs of designed siRNA respectively or in different combinations with lipofectamine 2000. Transfected NICC were analyzed for *TF* gene by real-time PCR to select the siRNA which worked best. Meanwhile, the viability of NICC after the TF siRNA transfection was examined by FACS. The efficiency of *TF* gene and protein suppression was measured by real-time PCR and and FACS respectively. **Results** Real-time PCR and FACS showed that a 60% reduction in the *TF* gene expression and a 50% reduction in the protien level of TF on NICC were achieved by transfecting 3 pairs of selected siRNA. The siRNA transfection had no significant effect on the viability of NICC which was analyzed by FACS. **Conclusion** The expression of TF on porcine NICC is efficiently suppressed by 3 pairs of designed siRNA in vitro.

Keywords: small interfering RNA tissue factor neonatal porcine islet cell cluster

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