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Title: Over-expression of lamin B1 in human immortal keratinocyte through lentiviral vector

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关键词: 人角质形成细胞; 慢病毒; 蛋白组学; 核纤层蛋白B1

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摘要: 目的 通过对慢病毒载体稳定转染人角质形成细胞 (HaCaT) 株和未转染HaCaT细胞进行比较蛋白组学分析, 找出其差异表达蛋白, 寻找基因修饰组织工程表皮种子细胞生长特性改变的原因和可能存在的成瘤性安全隐患。 方法 选择慢病毒载体稳定转染后生长特性发生明显改变的转染株细胞, 采用二维电泳技术对该转染株和未转染株的总蛋白进行分离和比较, 找出差异表达蛋白点, 再进行串联质谱鉴定。从鉴定得到的差异蛋白中选择可能与细胞生长特性改变和肿瘤生成相关的核纤层蛋白B1 (lamin B1) 采用Western blot和实时荧光定量PCR技术 (qPCR) 对其表达差异进行验证。 结果 与未转染株比较, 转染株在二维电泳中存在11个差异表达蛋白点, 质谱从其中鉴定出7个蛋白质。选取其中与细胞增殖和凋亡相关的核纤层蛋白B1通过WB和qPCR证实其在转染株中蛋白和转录水平均存在明显的高表达。 结论 慢病毒载体稳定转染株HaCaT细胞株相对未转染株核纤层蛋

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白B1高表达。这种高表达可能与转染株细胞生长特性改变和潜在的成瘤性隐患相关。

Abstract: **Objective** To determine the differences of human immortal keratinocyte line HaCaT with or without lentivirus-mediated CCL20 over-expression by proteomic analysis in order to investigate the cause of altered growth characteristics and potential threat of tumorigenicity in the seed cells for gene-modified tissue engineering. **Methods** Lentiviral vector (pHSER-CCL20-shRNA-GFP)-infected HaCaT cells with significant changes in growth characteristics and uninfected HaCaT cells were chosen as experimental and control cells, respectively. The differentially expressed proteins between them were revealed by two-dimensional electrophoresis, and these obtained proteins were further identified by LC-MS-MS. Lamin B1, which is closely related to tumorigenicity, cell growth and cell cycle, was picked out from these proteins and its differential expressions at protein and mRNA levels were validated by Western blotting and quantitative PCR (qPCR). **Results** Between these 2 cell lines, there were 11 differentially expressed proteins found by two-dimensional electrophoresis, and 7 proteins including lamin B1 identified from them by LC-MS-MS. Furthermore, increased expression of lamin B1 protein in lentiviral vector-infected HaCaT cells was proved by Western blotting as well as its mRNA expression by qPCR analysis. **Conclusion** An over-expression of lamin B1 protein is seen in lentiviral vector-infected human immortal keratinocyte, which may be correlated with altered growth characteristics of lentiviral vector-infected keratinocyte.

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