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缺氧/放射活化Epo/e9增强子调控layilinsiRNA表达以抑制人肺腺癌A549细胞侵袭的研究

Inhibitory effects of layilin siRNA expression regulated by Epo/e9 enhancer activated through hypoxia/radiation on invasion behavior of human lung carcinoma cell A549 induced by hyaluronan in vitro

中文关键词: Epo/e9增强子 layilin RNA干扰 侵袭 A549细胞

英文关键词: Layilin Epo/e9 enhancer RNA interference Invasion A549 cell line

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中文摘要：

背景与目的： Layilin是一种新发现与肺腺癌侵袭转移有密切联系的特异性透明质酸受体。本研究旨在观察缺氧/放射条件下活化缺氧/放射双敏感性增强子(Epo/e9)，调控针对layilin的小干扰RNA(small interfering RNA，siRNA)表达，探讨其对人肺腺癌A549细胞受透明质酸诱导侵袭的影响。**方法：** 构建携带Epo/e9增强子和U6启动子且表达针对layilin的siRNA的RNA干扰载体(Epo/e9-siLay plasmid)和阴性对照RNA干扰载体(Epo/e9-siCtrl plasmid)，分别转染A549细胞，新霉素抗性筛选得到layilin表达受抑制的$A549_{Epo/e9\text{-}siLay}$细胞和layilin表达未受影响的$A549_{Epo/e9\text{-}siCtrl}$细胞。针对非转染A549($A549_{untransfected}$)、$A549_{Epo/e9\text{-}siLay}$、$A549_{Epo/e9\text{-}siCtrl}$三组细胞，在缺氧/放射处理下，分别采用RT-PCR和Western blot检测layilin mRNA和蛋白表达，用Transwell模型检测细胞侵袭能力。**结果：** 与$A549_{untransfected}$组相比，$A549_{Epo/e9\text{-}siLay}$组layilin表达显著减弱($P<0.01$)，Transwell穿膜细胞数显著减少($P<0.01$)；$A549_{Epo/e9\text{-}siCtrl}$组layilin表达、Transwell穿膜细胞数皆和$A549_{untransfected}$组无显著差异($P>0.05$)。缺氧/放射处理能进一步增加RNA干扰的上述效应($P<0.01$)。**结论：** 在缺氧/放射条件下，Epo/e9增强子调控layilin siRNA表达能明显抑制A549细胞侵袭行为。

英文摘要：

Background and purpose: Layilin is a novel and special hyaluronan (HA) receptor that closely linked with lung cancer invasion and metastasis. The objective of this study is to investigate the effect of hypoxia/ radiation dual-sensitive enhancer (Epo/e9) regulating small interfering RNA (siRNA) for layilin expression on invasion of human lung adenocarcinoma A549 cells induced by HA in vitro. **Methods:** RNA interference plasmid including Epo/e9 enhancer and U6 promoter and expressing siRNA targeting layilin (Epo/e9-siLay plasmid) or expressing siRNA not matching any known human coding mRNA (Epo/e9-siCtrl plasmid) was designed, constructed, and lipotransfected into A549 cells line. $A549_{Epo/e9\text{-}siLay}$ cells expressing suppressed layilin or $A549_{Epo/e9\text{-}siCtrl}$ cells expressing uninfluenced layilin were selected by neomycin resistance. In $A549_{untransfected}$, $A549_{Epo/e9\text{-}siLay}$ and $A549_{Epo/e9\text{-}siCtrl}$ groups, layilin mRNA and protein expression were detected by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot, and the invasion ability was examined by Transwell model with or without administration of hypoxia/radiation. **Results:** Compared with that in $A549_{untransfected}$ group, layilin mRNA and protein expression, and the number of penetrating cells (NPC) were decreased significantly in $A549_{Epo/e9\text{-}siLay}$ group ($P<0.01$), but there were no significant changes in $A549_{Epo/e9\text{-}siCtrl}$ group ($P>0.05$). Administration of hypoxia/radiation reinforced the above effects of siRNA targeting layilin. **Conclusion:** Epo/e9 enhancer regulating siRNA for layilin expression can inhibit efficiently the invasive ability of human lung carcinoma cell line A549 induced by HA in vitro with hypoxia/radiation administration.

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