外源性S100A9通过TLR4诱导非小细胞肺癌细胞侵袭的实验研究

《现代肿瘤医学》[ISSN:1672-4992/CN:61-1415/R] 期数: 2019年09期 **页码:** 1508-1513 **栏目:** 论著（基础研究） **出版日期:** 2019-03-30

Title: **Experimental study of exogenous S100A9 induces cell invasion of non-small cell lung cancer by TLR4**

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关键词: S100A9; TLR4; 非小细胞肺癌; 细胞迁移

Keywords: S100A9; TLR4; non-small cell lung cancer; cell migration

分类号: R734.2

DOI: 10.3969/j.issn.1672-4992.2019.09.010

文献标识码: A

摘要: 目的：探讨外源性钙结合蛋白S100A9通过Toll样受体4（TLR4）诱导对非小细胞肺癌细胞H1650侵袭及迁移的影响。方法：收集非小细胞肺癌组织及癌旁组织，实验组：培养基添加1 μg/ml S100A9培养的H1650细胞，对照组：不添加任何血清蛋白培养的H1650细胞。RT-PCR和Western blot分别检测S100A9 mRNA和蛋白水平。RT-PCR检测转染后细胞中S100A9 mRNA的表达。采用划痕、黏附和平板克隆实验分别检测S100A9对H1650细胞生物学行为的影响，同时运用Western blot检测H1650细胞中TLR4蛋白，以观测其表达情况。结果：非小细胞肺癌组织中S100A9 mRNA和蛋白表达水平都高于癌旁组织，S100A9在非小细胞肺癌组织中过度表达(P<0.01)。S100A9小干扰RNA能够成功抑制非小细胞肺癌细胞中S100A9 mRNA水平(P<0.01)。与对照组相比，实验组加入S100A9蛋白后1 h，H1650细胞中TLR4的累积明显上调，S100A9起促进H1650细胞迁移(P<0.05或P<0.01)、基质黏附(P<0.01)和平板克隆(P<0.01)的作用。结论：TLR4在经过添加S100A9蛋白后NSCLC细胞株H1650细胞中的表达明显高于未添加S100A9蛋白的H1650细胞，有关实验中TLR4在肺癌中呈现为高表达，因此S100A9对肿瘤侵袭及迁移有着不可分割的联系，将成为肿瘤诊治的新靶点。

Abstract: Objective：To investigate the effects of exogenous S100A9 induced by TLR4 on H1650 cell invasion and migration of non-small cell lung cancer.Methods：Non-small cell lung cancer tissues were collected.In the experimental group，1 μg/ml S100A9 cultured H1650 was added to the culture medium，and the control group was not added with any serum protein-cultured H1650.RT-PCR and Western blot were used to detect the S100A9 mRNA and protein levels，respectively.The expression of S100A9 mRNA in the transfected cells was measured by RT-PCR.The effects of S100A9 on the biological behavior of H1650 cells were detected by scratch，adhesion and plate cloning experiments.Western blot was used to detect TLR4 protein in H1650 cells to observe its expression.Results：The expression of S100A9 mRNA and protein levels in non-small cell lung cancer was higher than that in the paracancerous tissue，and S100A9 was overexpressed in non-small cell lung cancer（P<0.01).S100A9 small interfering RNA can successfully inhibit S100A9 mRNA in non-small cell lung cancer cells（P<0.01).Compared with the control group，the accumulation of TLR4 in H1650 cells was significantly up-regulated 1 h after the addition of S100A9 protein in the experimental group.S100A9 also promoted the effects of H1650 cell migration（P<0.05/P<0.01)，matrix adhesion（P<0.01) and plate cloning（P<0.01).Conclusion：The expression of TLR4 in NSCLC cell line H1650 after S100A9 protein addition was significantly higher than that in H1650 tissue without S100A9 protein.In the related experiments,TLR4 was highly expressed in lung cancer,so S100A9 is inseparable for tumor invasion and migration.Contact will become a new target for cancer diagnosis and treatment.

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备注/Memo: 开封市科技发展计划项目(编号：1708007)

更新日期/Last Update: 2019-03-30