BDNF-AS基因及其突变体对HepG2细胞增殖和相关信号通路的影响

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Title: **Effects of BDNF-AS and its mutants on HepG2 cell proliferation and related signaling pathways**

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摘要: 目的：探讨BDNF-AS基因对肝癌细胞HepG2增殖的作用，以及对细胞内信号通路的影响。方法：采用BDNF-AS过表达质粒或BDNF-AS与BDNF基因完全互补序列缺失突变质粒转染肝癌细胞系HepG2，建立过表达BDNF-AS及其突变体的稳转细胞系，采用qRT-PCR方法检测HepG2细胞中BDNF-AS和BDNF mRNA水平，采用Western blot检测细胞中BDNF蛋白水平，采用EdU掺入实验评价细胞增殖情况，采用Western blot方法检测细胞内相关信号通路的活化情况。结果：稳定表达BDNF-AS及其突变体的细胞其BDNF-AS水平显著上调，而只有稳定表达BDNF-AS的细胞其BDNF mRNA水平显著下调，细胞增殖受到抑制。各组细胞BDNF蛋白水平无显著差异。各组细胞中PDK1和PKC底物的磷酸化水平上调，而过表达BDNF-AS组与突变组之间有差异。各组细胞中CK2底物的磷酸化水平下调，而过表达BDNF-AS组与突变组之间无显著差异。结论：HepG2细胞中稳定表达的BDNF-AS可能通过与BDNF基因的完全互补序列调控BDNF mRNA表达水平。稳定表达的BDNF-AS通过干扰CK2底物的磷酸化影响HepG2细胞的增殖能力，并且可能通过促进PDK1、PKC底物的磷酸化，影响HepG2细胞的其他生物学行为。

Abstract: Objective： To investigate the effect of BDNF-AS on proliferation of HepG2 and intracellular signaling pathways.Methods：Use BDNF-AS over-expression plasmid or complete complementary deletion mutant plasmid of BDNF to transfect HepG2，establish stable lines of over-expressed BDNF-AS and its mutants，use qRT-PCR to detect BDNF-AS and BDNF mRNA in HepG2，use Western blot to measure BDNF protein level，use EdU to evaluate the cell proliferation，use Western blot to detect the activation of relevant signaling pathways.Results：Cells with stable expression of BDNF-AS and its mutants had significantly up-regulated of BDNF-AS，but cells with stable expression of BDNF-AS had significantly down-regulated of BDNF mRNA，and the cell proliferation was inhibited.There was no significant difference in BDNF protein level in each group.The phosphorylation levels of PDK1 and PKC substrates were up-regulated in each group，and there was a difference between the over-expressed BDNF and the mutants.The phosphorylation levels of CK2 substrates were down-regulated in each group，and there was no difference between the over-expressed BDNF-AS and the mutants.Conclusion：The stable expression of BDNF-AS in HepG2 may through the complete complement sequence of the two genes to regulate the BDNF mRNA.The stable expression of BDNF-AS can affect the ability of multiply by interfering the phosphorylation of CK2 substrates，may through promoting PDK1,PKC substrates phosphorylation to affect other biological behavior of HepG2.

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