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论著

人膀胱移行细胞癌EGFR基因片段与核基质蛋白的相关性

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摘要 背景与目的： 研究人膀胱移行细胞癌中表皮生长因子受体(epidermal growth factor receptor, EGFR)基因片断与核基质蛋白(nuclear matrix proteins, NMPs)的相关性。 材料与方法： 分别提取培养细胞和膀胱癌组织标本的核基质蛋白及其全细胞蛋白，同时抽提组织标本的基因组DNA和核基质DNA，根据EGFR cDNA序列合成两对PCR引物，分别以基因组DNA和各核基质DNA为模板，PCR扩增其结合片断；并对引物Ⅰ产物进行序列测定；以引物Ⅱ的产物制备探针，southwestern 杂交进一步检测EGFR基因片断与核基质蛋白结合情况。 结果：110 bp PCR(引物Ⅱ)产物见于基因组DNA和各核基质DNA (NM DNA 0 , NM DNA 25 , NM DNA 50 , NM DNA 100)；940 bp PCR(引物Ⅰ)产物出现在基因组DNA和除NM DNA100外其他核基质DNA中。940 bp序列与EGFR基因DNA序列完全一致。 Southwestern blot检测结果表明，全细胞蛋白及核基质蛋白中出现特异的分子量约为60 kD的DNA-蛋白阳性杂交条带。 结论： 人膀胱移行细胞癌中，EGFR活性转录基因片段与核基质及核基质蛋白结合，NMPs可能和EGFR基因转录或转录后翻译等基因调控事件相关，核基质对EGFR在膀胱癌中高表达可能具有重要的调控作用。

关键词 表皮生长因子受体基因； 核基质蛋白； 膀胱移行细胞癌

Correlation between EGFR Gene Fragments with Nuclear Matrix Proteins in Bladder Cancer

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Abstract BACKGROUND & AIM: To study the correlation between EGFR gene fragments and nuclear matrix proteins (NMPs) in human bladder transitional cell carcinoma (hBTCC). MATERIALS AND METHODS: The nuclear matrix proteins were prepared from fresh tissues and T24 culture cells, and total cell proteins was also prepared from fresh tissues of hBTCC. Genomic DNA and nuclear matrix DNA (high concentration sail and gradient DNase Ⅰ treated) were separated from hBTCC tissues. Two pair primers were synthesized according to the cDNA sequence of EGFR gene.Two EGFR fragments were amplified by PCR using the two pair primers in genomic DNA and different nuclear matrix DNA.The positive fragments with primers Ⅰ were also sequenced. The binding capacity of EGFR gene to nuclear matrix in bladder cancer was also measured by Southwestern Blot assay using single stranded end labeled probes prepared by PCR product Ⅱ. RESULTS: Both genomic DNA and nuclear matrix DNA (NM DNA0, NM DNA25 ,NM DNA50 ,NM DNA100) of human bladder carcinoma could amplify 110 bp positive fragment with primers Ⅱ. However both genomic DNA and nuclear matrix DNA showed a 940 bp positive fragment with primers Ⅰ except NM DNA100. The sequence of 940 bp was identical to the DNA sequence of EGFR gene. Southwestern blot analysis demonstrated that EGFR gene fragment (3901-4010nt) was bound to an about 60 kD nuclear matrix protein in both NMPs (tissues and T24 cells) and total cell protein of bladder carcinoma tissues, but not in the cytoplasm. CONCLUSION: Active EGFR gene is strongly bound to the nuclear matrix and nuclear matrix proteins.Nuclear matrix proteins may regulate the high expression of EGFR gene

including the course of transcription and post-transcription and/or translation in the human bladder transitional cell carcinoma.

Keywords epidermal growth factor receptor nuclear matrix proteins bladder transitional cell carcinoma

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