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Construction of Prostate-Specific Expressed Recombinant Plasmids With High Transcriptional Activity of Prostate-Specific Membrane Antigen (PSMA) Promoter/Enhancer

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To screen different combinations of prostate-specific membrane antigen (PSMA) promoter/enhancer with the strongest transcriptional activity in prostate-specific cells, we used PSMA regulatory elements to control specific expression of the target gene in gene therapy of prostate adenocarcinoma. PSMA promoter and enhancer DNA sequences were amplified from the LNCaP human prostate cancer cell line by polymerase chain reaction, then recombinant plasmids of the enhanced green fluorescent protein (EGFP: pEGFP-$PSMA_{Pro}$, pEGFP-$PSMA_{E\text{-}P}$, pEGFP-$PSMA_{E(r)\text{-}P}$, pEGFP-$PSMA_{E(d)\text{-}P}$, and pEGFP-$PSMA_{E(t)\text{-}P}$) were constructed with molecular clonal techniques. At the same time, all experimental cell lines were analyzed for the expression of PSMA with the use of PSMA monoclonal antibody and the ABC immunohistochemical assay kit. After plasmids were transfected via liposome, we observed the expression of the reporter gene (EGFP) under a fluorescent microscope and compared the different levels of EGFP expression with reverse transcriptase polymerase chain reaction and flow cytometry so that we could choose the one with the highest transcriptional activity. Only the LNCaP cell line expressed PSMA positively with immunohistochemical stain. The PSMA promoter/enhancer had transcriptional activity in PSMA(+) cell lines and no activity in PSMA(-) cell lines. $PSMA_{E\text{-}P}$ achieved the strongest activity in different PSMA promoter/enhancer combinations. We confirmed the specific expression of PSMA in prostate cells again. Similarly, transcriptional activity of the PSMA promoter/enhancer was prostate specific. $PSMA_{E\text{-}P}$ achieved the strongest transcriptional activity among PSMA promoter/enhancer combinations, which could be used in advanced research for tissue-specific treatment.

Key words: Adenocarcinoma, regulatory element

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