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MAGE-1 cDNA ISOLATION FROM TESTIS WITH RT PCR

Abstract

Hepatocellular carcinoma (HCC) is one of the most common malignancies in Asia and it is generally diagnosed at advanced stage, during which the prognosis of the patients has been poor and their capability to survive has also been low. Hepatic tissue of HCC patients expresses melanoma antigen-1 (MAGE-1) gene in the form of mRNA that can be identified only by RT PCT examination. mRNA examination with RT PCR is relatively more complicated since RNA is easier to be degraded by RNAase, resulting in difficulties in isolating the gene cDNA. Optimized examination with RT PCR is needed to be able to isolate MAGE-1 cDNA from testicular tissue that definitely expresses MAGE-1 mRNA. However, to date RT PCR optimal RT PCR from testis samples has never been undertaken. This was a laboratory explorative study generally aimed to isolate MAGE-1 cDNA from testicular tissue with RT PCR. The particular objective of this study was to obtain optimum condition for isolating cDNA of MAGE-1 gene from testicular tissue using RT PCR and to obtain MAGE-1 cDNA sequence from testicular tissue. mRNA isolation of MAGE-1 gene was obtained by sample extraction to obtain total RNA, which was subsequently altered into cDNA by means of oligo(T) polymer. Formed cDNA was amplified with polymerase chain reaction (PCR) using the primers GM F421 and GM R421 that synthesized 421bp. PCR product was electrophoresized and visualized with UV transluminator. PCR product was also sequenced with Genetic Analyzer ABI Prism 310. The result showed that nucleotide sequence of MAGE-1 gene was accessible in Genbank with access number of EU161102. Optimum condition to obtain MAGE-1 cDNA from testicular tissue was predenaturation in 95°C for 5 minutes, denaturation in 95°C for 1 minute, annealing at 58°C or 60°C for 1 minute, extension at 72°C for 1 minute, 35 cycles. The cycle was prolonged at 72°C for 10 minutes. In conclusion, cDNA isolation of MAGE-1 gene can be obtained by using RT PCR from testicular tissue. The result of this study can be used as a basis for further studies in exploring the expression of MAGE-1 gene from Hepatocellular carcinoma (HCC) patient with RT PCR.

Keyword : testis, MAGE-1, RT, PCR,