MOLECULAR ANALYSIS OF THE CODING SEQUENCE OF MAGE-1 GENE FROM HEPATOCELULAR CARCINOMA PATIENT AND TESTIS

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ABSTRACT

The gene MAGE-1 is expressed approximately 60-80% in hepatocellular carcinoma. The gene is also expressed in normal testis. However, molecular analysis of coding area of the gene MAGE-1 from liver tissue of hepatocellular carcinoma patients and normal testicular tissue remains unclear. The objective of this study was to conduct molecular analysis on the coding area of the gene MAGE-1 from liver tissue of hepatocellular carcinoma patients and normal testicular tissue. This was a laboratory explorative study. Samples were obtained from the result of FNAB of the liver tissue from hepatocellular carcinoma patients and normal testicular tissue. RNA was extracted from the sample and then subjected to cDNA synthesis using oligo-T primer. cDNA was used as template for nested PCR. Afterwards, the PCR product was sequenced. Molecular analysis of coding area of gene MAGE-1 was conducted using Clone Manager software and using the site European Bioinformatic Institute http://www.ebi.ac.uk/Tools/clustalw2. Homology analysis of coding area sequence of gene MAGE-1 from normal testicular tissue and the sequence from liver tissue of hepatocellular carcinoma patients had a homology of 100%. The coding sequence of gene MAGE-1 from liver tissue of hepatocellular carcinoma patients and normal testicular tissue had a homology of 100% with sequence accession number M77481, NM_004988, and 99% with sequence accession number BC017555 and AY148486. The whole area of gene MAGE-1 coding could be isolated from the result of FNAB from the liver tissue of patients with hepatocellular carcinoma and those with normal testis using nester PCR, whose the final result presented as a band of +931 pb, containing 927 nucleotides that coded 309 amino acid. The coding area sequence of gene MAGE-1 coding in the liver tissue of hepatocellular carcinoma patients is the same to that in normal testicular tissue (100% homologous).

Keywords: *MAGE-1, molecular analysis, hepatocellular carcinoma, normal testicular tissue*

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INTRODUCTION

Melanoma antigen (MAGE) gene belongs to testicular cancer gene and it is designated in such a way because it is only expressed in cancer cells and normal testis. The term testicular-cancer gene is used to refer coding gene and testicular-cancer antigen is used to refer the antigen (Van Baren et al, 1999; Luo et al, 2002). MAGE-1 gene includes MAGE gene, comprising 3 exons and 2 introns. The whole coding areas of MAGE-1 gene that code MAGE-1 protein are located in exon 3, consisting of 930 bp (base pair). According to several studies, MAGE-1 gene is most frequently expressed in hepatocellular carcinoma, consisting 60-80% (Chen et al, 2000; Kariyama et al, 1999; Liu et al, 1999; Miyamoto et al, 2000; Mou et al, 2002; Peng et al, 2002; Zhao et al, 2004). According to Van Baren et al, (1999) dan Zhao L, et al, (2004), MAGE-1 gene is also expressed in normal testis, but molecular analysis of MAGE-1 gene coding area from liver tissue of hepatocellular carcinoma patients and normal testicular tissue remained unclear.

Hepatocellular carcinoma is a cancer with the fifth highest incidence in the world and the third cancer-related cause of deaths. The percentage of mortality is about 97% of the total incidence. There are more than 600,000 new cases of hepatocellular carcinoma diagnosed every year (Mathers et al, 2001). According to WHO data, Indonesia is a high risk area with incidence occupying the fifth rank of the whole cancer incidence and the fourth cancer-related causes of death

(WHO 2004). The beginning of hepatocellular carcinoma process is not accompanied with clear sign and symptoms, so that the patient does not realize until the tumor has reached a wide size in advanced stage. Therefore, general prognosis of hepatocellular carcinoma always poor and the patient finally dies. The remaining survival time is 6 months after diagnosis and 11 weeks after the emergence of the symptom (Lopez 2005). Diagnosis was established based on physical examination followed with other procedures, including ultrasonography (USG), computed tomography (CT), magnetic resonance imaging (MRI), or CT angiography (Okita 2006). Examination using those techniques was based on tissue structural change in which the cancer can be detected after it becomes enlarged. Therefore, the diagnosis can only be established after it reaches advance stage. Laboratory examination that may be helpful in the diagnosis is Alpha-fetoprotein (AFP) level examination (Soemohardjo 2004). However, not all patients demonstrate increasing level of AFP (El-Hoseini et al, 2005). Additionally, AFP is also secreted by normal liver cells and infected by hepatitis virus, so that the increase of AFP level does not necessarily indicate a malignancy (Mou et al. 2002; Yang et al. 2005). Other examinations are PIVKA II (protein induced vitamin K absent/antagonis II) and osteopuntin. PIVKA II examination is often used for hepatocellular carcinoma diagnosis, but remains less sensitive since PIVKA II is also synthesized by normal liver cells and liver cells infected with hepatitic virus (Suehiro et al. 1994). Osteopuntin is a glycoprotein synthesized in physiological and pathological condition by proeosteoblast, osteoblast, osteocyte, macrophage, smooth muscle, and endothelial cells (Benhadt et al. 2001). Therefore, both examinations do not fully reflect the presence of hepatocellular carcinoma. Another examination is the Gamma Glutamyl Tranfererase (GGT). However, this examination is also less specific since GGT level also increases in viral hepatitic and fatty liver (Muliawan 2000). The examination of Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic-Pyruvic Transaminase (SGPT) are for examining hepatic function that does not reflect changes toward malignancy. Some of those examinations are less specific to identify malignancy in the liver. Based on those facts, more specific examination for hepatic malignancy is still required to detect hepatocellular carcinoma.

MAGE-1 gene is expressed in the tumor with a size of less than 2 cm and in normal or abnormal AFP levels, and not expressed in non-tumor liver cells and the ones infected with hepatitic virus (Kobayashi et al, 2000). MAGE-1 gene expression can be used to predict the occurrence of hepatocellular carcinoma in high-risk patients, so that it can be employed as a basis for establishing the diagnosis of the patients. This gene expression occurs at the onset of carcinogenesis, so that it can be used as an early diagnosis of hepatocellular carcinoma (Yang et al. 2005). Such expression can be examined from liver tissue of hepatocellular carcinoma patients. There has been obstacles to obtain sample examination from the liver tissue of those patients because open biopsy and liver tissue resection in hepatocellular carcinoma patients are rarely conducted because of uncontrolled bleeding risk. It is therefore difficult to carry out MAGE-1 gene expression examination from liver tissue of those patients. According to Takano et al. (1999) and Giardia et al. (2002), gene expression examination can be conducted from samples obtained using closed fine-needle aspiration biopsy (FNAB), so that the samples from FNAB can be used as alternative for isolating coding area of MAGE-1 gene, allowing molecular analysis of the gene.

According to Van Baren et al. (1999) and Zhao et al. (2004) MAGE-1 gene is also expressed in normal testis. MAGE-1 gene coding area sequence from testicular tissue and liver tissue of patients with hepatocellular carcinoma should be analyzed. If there are sequence similarities with high level of homology, the sequence of testicular tissue can be used as samples for making hepatocellular carcinoma diagnostic kit. The sample of testicular tissue was obtained from prostate cancer patients of more than 70 years old who received orchidectomy therapy, while the sample of liver tissue can be obtained from the result of FNAB of the liver tissue in hepatocellular carcinoma patients. Sampling using FNAB technique was carried out by means of small gauge needle (22 or 24 G) guided with CT Scan. Therefore, it was necessary to perform molecular analysis of MAGE-1 gene coding area from the liver tissue of hepatocellular carcinoma patients and testicular tissue. The general objective of this study was to perform molecular analysis of MAGE-1 gene coding area from the liver tissue of hepatocellular carcinoma patients and testicular tissue. The particular objective in this study was to isolate MAGE-1 gene coding area cDNA from the liver tissue of hepatocellular carcinoma patients and testicular tissue, to obtain MAGE-1 gene coding area sequence from the liver tissue of hepatocellular carcinoma patients and testicular tissue, and to perform homology analysis of MAGE-1 gene coding area sequence from the liver tissue of hepatocellular carcinoma patients and testicular tissue.

MATERIALS AND METHOD

This was an explorative laboratory study. Samples were obtained from the FNAB results of the liver tissue of

hepatocellular carcinoma patients and normal testicular tissue of prostate cancer patients aged more 70 years who received orchidectomy. Sampling was performed using FNAB with 22G needle directed to the nodule in the liver assisted with CT Scan. Sample preparation from the FNAB was conducted as follows: cells within the needle was inserted within a tube containing RLT and ME buffer from RNeasy Protect Mini kit (Qiagen) and the reaspirated using the same needle and sprayed again into the tube. The procedure was repeated five times to carry out cell lysis in the needle. Sample solution was kept in 40 degree C until its use in the subsequent process. In taking samples from normal testicular tissue, the tissue was separated from testis-enveloping tunic, and the tissue was taken of about 30 mg (3x3x3 mm) and placed within sterile Eppendorf tube containing 500 ul RNAlater RNA stabilization Reagent (Qiagen) for stabilizing the RNA. The tissue was kept in 40 degree C until its use int he subsequent process.

RNA extraction was performed using RNeasy Protect Mini Kit (Qiagen). cDNA synthesis was performed using First strand cDNA Syntesis kit for RT-PCR (AMV) (Roche). Primer for cDNA synthesis was oligo-T. All procedures were performed according to the protocols of each reagent used. cDNA was used as template for PCR. PCR was performed with High Fidelity Platinum Taq DNA Polymerase (Invitrogen) kit with nested PCR. Primer pair for first round PCR was primer forward GM421F 5'-CGG CCG AAG GAA CCT GAC CCA G-3' and GMTOPOR 5'-GCT TTG AGA GAG GAG GAA GAG GGA GTC-3' that produced about 1105 pb. PCR product from the first round was used as template for second round PCR using the primer pair GMTOPOF 5'-CACC ATG TCT CTT GAG CAG AGG AGTC-3' for forward and GMTOPOR 5'-GCT TTG AGA GAG GAG GAA GAG GGA GTC-3' for reverse that produced 931 pb. PCR condition was as follows: predenaturation in 95 degree C for 5 minutes, denaturation in 95 degree C for 1 minute, annealing in 60 degree C 1 minute, extension in 72 degree C for 1 minute, 35 cycles and final extension in 72 degree C for 10 minutes. The result of PCR was analyzed with electrophoresis gel in 2% agartose gel containing ethidium bromide. An amount of 5 µl DNA added with 2 µl loading dye was put within agarose wells, and run in 100 volt for about 30 minutes, and then detected with UV-transilluminator and photographed with polaroid camera. PCR product resulted was purified earlier for sequencing using Gel PCR Purification kit (Qiagen). Sequencing was conducted with DNA sequencer (ABI Prism 310). Molecular analysis of MAGE-1 gene coding area sequence from the liver tissue of hepatocellular carcinoma patients and testicular tissue was performed using Software Clone Manager and using the site European Bioinformatic Institute http://www.ebi.ac.uk/Tools/clustalw2/.

RESULTS

RNA extraction was performed to obtain total RNA which was subsequently used for cDNA synthesis. cDNA synthesis was carried out using oligo T primer. There was poly A at the 3' end of mRNA, so that oligo T would be paired with the poly A. Then, cDNA synthesis occurred with the help from reverse transcriptase enzyme. The formed cDNA was used as a template for PCR. MAGE-1 gene coding area amplification from the result of FNAB from the liver tissue for hepatocellular carcinoma patients and normal testicular tissue was performed with nested PCR. Primer pairs for first round PCR were the forward primer GM421F 5'-CGG CCG AAG GAA CCT GAC CCA G-3' and the reverse primer GMTOPOR 5'-GCT TTG AGA GAG GAG GAA GAG GGA GTC-3' that produced product of 1105 pb. PCR product from the first round was used as template for second round PCR using the primer pair GMTOPOF 5'-CACC ATG TCT CTT GAG CAG AGG AGTC-3' for forward and GMTOPOR 5'-GCT TTG AGA GAG GAG GAA GAG GGA GTC-3' for reverse, producing 931 pb that amplified all MAGE-1 gene coding areas present in exon 3. Based on the result of PCR condition optimation used in this study, predenaturation was undertaken in 95 degree C for 5 minutes 1 cycle, denaturation in 95 degree C for 1 minute, annealing in 58 degree C for 1 minute and extension in 72 degree C for 10 minutes. The result of the first round using primers GM421F and GMTOPOR revealed bands of 1105 pb. The result of the second round using primers GMTOPOF and GMTOPOR showed band of 931 pb. PCR product was electrophoresized and visualized with UV-transilluminator and documented with polaroid camera as seen in Figures 1 and 2.

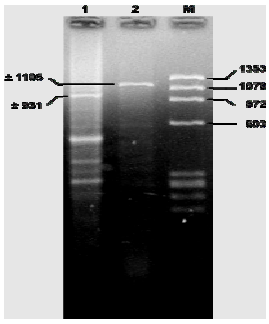

Figure 1. The result of nester PCR electrophoresis of MAGE-1 gene coding area from normal testicular tissue.

1 = second round PCR product (+ 931bp), 2 = first round PCR product (+ 1105 bp), M = Marker (x 174 RF DNA/Hae III fragments).

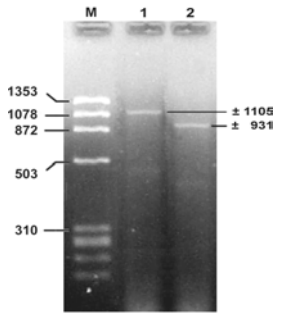

Figure 2. The result of nester PCR electrophoresis of MAGE-1 gene coding area from the FNAB of the liver tissue of hepatocellular carcinoma patients. 1 = first round PCR product (+ 1105 bp), 2 = second round PCR product (+ 931bp), M= Marker (x 174 RF DNA /Hae III fragments).

PCR product was purified and sequenced. Sequencing was undertaken using DNA sequencer machine ABI PRISM 310. The result of sequencing of MAGE-1 gene coding area sequence data from the FNAB of liver tissue from hepatocellular carcinoma patients and normal testicular tissue was analyzed using Clone Manager Software. The results of sequencing indicated that MAGE-1 gene coding area from the result of FNAB from the liver tissue of hepatocellular carcinoma patients and normal testicular tissue consisted of 927 nucleotides coding 309 amino acids, including start codon indicated in Figure 3 and 4.

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  1  ATGTCTCTTG AGCAGAGGAG TCTGCACTGC AAGCCTGAGG AAGCCCTTGA GGCCCAACAA
 61  GAGGCCCTGG GCCTGGTGTG TGTGCAGGCT GCCACCTCCT CCTCCTCTCC TCTGGTCCTG
121  GGCACCCTGG AGGAGGTGCC CACTGCTGGG TCAACAGATC CTCCCCAGAG TCCTCAGGGA
181  GCCTCCGCCT TTCCCACTAC CATCAACTTC ACTCGACAGA GGCAACCCAG TGAGGGTTCC
241  AGCAGCCGTG AAGAGGAGGG GCCAAGCACC TCTTGTATCC TGGAGTCCTT GTTCCGAGCA
301  GTAATCACTA AGAAGGTGGC TGATTTGGTT GGTTTTCTGC TCCTCAAATA TCGAGCCAGG
361  GAGCCAGTCA CAAAGGCAGA AATGCTGGAG AGTGTCATCA AAAATTACAA GCACTGTTTT
421  CCTGAGATCT TCGGCAAAGC CTCTGAGTCC TTGCAGCTGG TCTTTGGCAT TGACGTGAAG
481  GAAGCAGACC CCACCGGCCA CTCCTATGTC CTTGTCACCT GCCTAGGTCT CTCCTATGAT
541  GGCCTGCTGG GTGATAATCA GATCATGCCC AAGACAGGCT TCCTGATAAT TGTCCTGGTC
601  ATGATTGCAA TGGAGGGCGG CCATGCTCCT GAGGAGGAAA TCTGGGAGGA GCTGAGTGTG
661  ATGGAGGTGT ATGATGGGAG GGAGCACAGT GCCTATGGGG AGCCCAGGAA GCTGCTCACC
721  CAAGATTTGG TGCAGGAAAA GTACCTGGAG TACCGGCAGG TGCCGGACAG TGATCCCGCA
781  CGCTATGAGT TCCTGTGGGG TCCAAGGGCC CTCGCTGAAA CCAGCTATGT GAAAGTCCTT
841  GAGTATGTGA TCAAGGTCAG TGCAAGAGTT CGCTTTTTCT TCCCATCCCT GCGTGAAGCA
901  GCTTTGAGAG AGGAGGAAGA GGGAGTC
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Figure 3. Results of MAGE-1 gene coding area sequencing of the samples from FNAB of liver tissue of hepatocellular carcinoma patients.

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  1  ATGTCTCTTG AGCAGAGGAG TCTGCACTGC AAGCCTGAGG AAGCCCTTGA GGCCCAACAA
 61  GAGGCCCTGG GCCTGGTGTG TGTGCAGGCT GCCACCTCCT CCTCCTCTCC TCTGGTCCTG
121  GGCACCCTGG AGGAGGTGCC CACTGCTGGG TCAACAGATC CTCCCCAGAG TCCTCAGGGA
181  GCCTCCGCCT TTCCCACTAC CATCAACTTC ACTCGACAGA GGCAACCCAG TGAGGGTTCC
241  AGCAGCCGTG AAGAGGAGGG GCCAAGCACC TCTTGTATCC TGGAGTCCTT GTTCCGAGCA
301  GTAATCACTA AGAAGGTGGC TGATTTGGTT GGTTTTCTGC TCCTCAAATA TCGAGCCAGG
361  GAGCCAGTCA CAAAGGCAGA AATGCTGGAG AGTGTCATCA AAAATTACAA GCACTGTTTT
421  CCTGAGATCT TCGGCAAAGC CTCTGAGTCC TTGCAGCTGG TCTTTGGCAT TGACGTGAAG
481  GAAGCAGACC CCACCGGCCA CTCCTATGTC CTTGTCACCT GCCTAGGTCT CTCCTATGAT
541  GGCCTGCTGG GTGATAATCA GATCATGCCC AAGACAGGCT TCCTGATAAT TGTCCTGGTC
601  ATGATTGCAA TGGAGGGCGG CCATGCTCCT GAGGAGGAAA TCTGGGAGGA GCTGAGTGTG
661  ATGGAGGTGT ATGATGGGAG GGAGCACAGT GCCTATGGGG AGCCCAGGAA GCTGCTCACC
721  CAAGATTTGG TGCAGGAAAA GTACCTGGAG TACCGGCAGG TGCCGGACAG TGATCCCGCA
781  CGCTATGAGT TCCTGTGGGG TCCAAGGGCC CTCGCTGAAA CCAGCTATGT GAAAGTCCTT
841  GAGTATGTGA TCAAGGTCAG TGCAAGAGTT CGCTTTTTCT TCCCATCCCT GCGTGAAGCA
901  GCTTTGAGAG AGGAGGAAGA GGGAGTC
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Figure 4. Results of MAGE-1 gene coding area sequencing of the samples from normal testicular tissue.

Homology analysis was conducted on the site European Bioinformatic Institute http://www.ebi.ac.uk/Tools/clustalw2/. Sequence analysis of the sequence from FNAB of the liver tissue of hepatocellular carcinoma patients and normal testicular tissue indicated a 100% homology. The homology analysis of MAGE-1 coding gene sequence in this study with the sequence in GeneBank showed that sequence from normal testicular tissue (the testis) and sequence from liver tissue of hepatocellular carcinoma patients also had 100% homology (Table 1). MAGE-1 coding gene sequence from samples of normal testicular tissue had 100% homology with sequence accession number M77481, NM_004988 dan 99% with sequence accession number BC017555 and AY148486. Sequence from liver tissue of hepatocellular carcinoma patients had 100% homology with the sequence with accession numbers M77481, and, NM_004988, and 99% with sequence accession numbers BC017555 and AY148486. The sequence BC017555 and AY148486 had 100% homology, and the sequence M77481 was 100% homologue with the sequence NM_004988. Sequences with accession numbers BC017555, M77481, and NM_004988 were derived from the skin of melanoma patients and sequences with accession numbers AY148486 and AF463515 were from the liver tissue of hepatocellular carcinoma patientes. Sequence with accession number of AF463515 was an incomplete partical sequence, which was not used in homology analysis.

Table 1. Homology analysis of sequencing results in this study and the sequence existing in GeneBank

SeqA	Name	Len(nt)	SeqB	Name	Len(nt)	Score
1	testis	930	2	liver	930	100
1	testis	930	3	BC017555	930	99
1	testis	930	4	AY148486	930	99
1	testis	930	5	M77481	930	100
1	testis	930	6	NM_004988	930	100
2	liver	930	3	BC017555	930	99
2	liver	930	4	AY148486	930	99
2	liver	930	5	M77481	930	100
2	liver	930	6	NM_004988	930	100
3	BC017555	930	4	AY148486	930	100
3	BC017555	930	5	M77481	930	99
3	BC017555	930	6	NM_004988	930	99
4	AY148486	930	5	M77481	930	99
4	AY148486	930	6	NM_004988	930	99
5	M77481	930	6	NM_004988	930	100

Homology analysis of predicted amino acid is shown in Figure 6. The result indicates similarities between MAGE-1 amino acid in sample from normal testicular tissue and that from liver tissu eofo hepatocellular carcinoma patients. Similarity of amino acid was also found in MAGE-1 amino acid from skin tissue of melanoma patient (M77481 and NM_004988) and there was one amino acid different between melanoma skin tissue (BC017555) and liver tissue of hepatocellular carcinoma patients (AY148486).

DISCUSSION

Cloned MAGE-1 gene coding area is located on exon 3, between nucleotide 188 to 1117. MAGE-1 gene coding area amplification was conducted using nested PCR technique. Such technique was chosen because PCR can be performed twice using two pairs of primer. The primer GM421F attaches to exon 1 of MAGE-1 coding gene, the most varied area for MAGE family and the most conserved for MAGE-1, so that it could be employed as forward primer for the first round PCR. Reverse primer employed in this study was the GMTOPOR, which produced 1105 pb. Primer pair GM421F and GMTOPOR is highly specific for MAGE-1 mRNA. The PCR product was used as a template for the second PCR (second round) using the primer pairs GMTOPOF and GMTOPOR that lifted all MAGE-1 coding gene coding areas. The second PCR produced 931 pb.

Figure 5. Results of MAGE-1 gene homology analysis from normal testicular tissue and sample from FNAB of liver tissue from hepatocellular carcinoma patients.

Based on the result of optimum PCR condition in this study, predenaturation was performed in 95 degree C for 5 minutes 1 cycle, denaturation in 95 degree C for 1 minute, annealing 58 degree C for 1 minute and extension 72 degree C for 1 minute, for 31 cycles, and final extension was in 72 degree C for 10 minutes. The PCR product was electrophoresized and visualized with UV-transiluminator and documented with polaroid camera. The result of nested PCR electrophoresis of MAGE-1 gene coding area from FNAB of the liver tissue from hepatocellular carcinoma patients and normal testicular tissue and normal testicular tissue as shown in Figure 1 and 2.

Based on such result, the whole MAGE-1 gene coding areas could be isolated using nested PCR technique, whose end result presented as band of + 931 pb. Confirmation of MAGE-1 gene coding area sequence can be obtained from the sequencing. The result of bioinformatics analysis to obtain MAGE-1 gene coding area sequence data from FNAB of the liver tissue of hepatocellular carcinoma patients and samples of normal testicular tissue using Software Clone Manager are shown in Figure 3 and 4. The result of sequencing

indicated that MAGE-1 coding gene from normal testicular tissue and FNAB from liver tissue of hepatocellular carcinoma patients and FNAB of the liver tissue from hepatocellular carcinoma patients comprised 927 nucleotides that encode 309 amino acids, including start and without stop codon. This was in line with the data of MAGE-1 gene present in GeneBank, in which MAGE-1 consisted of 3 exons and 2 introns. MAGE-1 mRNA consisted of 1722 nucleotides, where the coding area is located in exon 3 in nucleotides 188-1117 (927 nucleotides including start and without stop codon). After the stop codon (nucleotide 1117), there remained several nucleotides and poly tail A.

The result of bioinformatics analysis to obtain MAGE-1 gene sequence data in GeneBank was performed in the site http://www.ncbi.nlm.nih.gov/sites/ entrez ?db=nuccore. The obtained data were 5 MAGE-1 mRNA sequences with accession numbers BC017555, AY148486, M77481, AF463515, and NM_004988, and 2 genome DNA sequence Xq28 chromosome with accession numbers AC152005 and AC153070. Sequence with accession number AF463515 is a incomplete partial sequence, not used in the subsequent analysis.

Homology analysis was performed in the site European Bioinformatic Institute http://www.ebi.ac.uk/Tools/clustalw2/. MAGE-1 gene coding are sequence used as control was the sequence from AC152005 and AC153070, which were genomic DNA sequence of Xq28 chromosome present in GeneBank. The objective of this homology analysis was to find the level of nucleotide similarity and difference between several sequences in comparison. In addition to the nucleotide, homology analysis can also be performed to amino acid. Amino acid has a wobble characteristic, in which it can be encoded by several codon codes, so that one nucleotide difference sometimes may result in different amino acid, but may also result in no change in the amino acid.

The result of homology analysis of MAGE-1 gene coding area sequence from FNAB of the liver tissue of hepatocellular carcinoma patients and samples from normal testicular tissue showed 100% homology, as can be seen in Figure 5. This indicates that nucleotide sequence of MAGE-1 coding gene present in liver tissue of hepatocellular carcinoma patient and normal testicular tissue is the same. The result of this study is useful for demonstrating that there is no difference in the nucleotide of MAGE-1 coding gene from normal testicular tissue and liver tissue of hepatocellular carcinoma patient, so that if it is difficulat to isolate MAGE-1 coding gene from liver tissue of hepatocellular carcinoma patient, isolation can be performed from normal testicular tissue by means of orchidectomy.

The result of homology analysis of MAGE-1 gene coding are sequence in this study and the sequence present in GeneBank is displayed in Table 1. The result shows that the sequence of normal testicular tissue and the sequence from the liver of hepatocellular carcinoma patient had 100% homology. The result showed that MAGE-1 coding gene sequence from normal testicular tissue and liver tissue of carcinoma patient is the same, indicating no difference in nucleotide and amino acid.

The sequence of MAGE-1 coding gene from normal testicular tissue samples and liver tissue of hepatocellular carcinoma patient had 100% homology with sequence accession number M77481 (sequence from melanoma skin tissue), NM_004988 (sequence from melanoma skin tissue) and 99% with sequence accession number BC017555 (sequence dari jaringan kulit melanoma) and AY148486 (from the liver tissue of hepatocellular carcinoma patients). This indicates that MAGE-1 coding gene sequence in this study with sequence in GeneBank from melanoma skin tissue (M77481 and NM_004988) are the same, but has one nucleotide difference from the sequence from melanoma skin tissue (BC017555) and the sequence from the liver of hepatocellular carcinoma patients (AY148486). One nucleotide difference is a type of polymorphism as that reported by Wang LP et al (2004) who found three types of nucleotide sequences of MAGE-1 coding gene from liver tissue of hepatocellular carcinoma patients, accessible in GeneBank with accession number AY148486 and AF463515, and one sequence as the same as that in GeneBank with accession number M77481. There was alteration in three nucleotides in the sequence AY148486, which was different from the sequence M77481, i.e., A272G, C991T, and A1125G, which lead to the alteration of 1 amino acid, the T32A. Whereas, AF463515 had three nucleotide difference, C159T, A272G, and G393A, that lead to alteration of 2 amino acids, the T32A and R72Q. Sequence with accession number M77481 was the same with the sequence of DNA genome.

Homology level of this nucleotide had not been able to reflect the level of alteration of protein-forming aminoc acid since different codon may produce the same amino acid, so that the homology analysis of the amino acid was performed. The result of homology analysis of predicted amino acid is shown in Figure 6. The result shows that predicted MAGE-1 amino acid sequence from normal testicular tissue and the liver tissue of hepatocellular carcinoma patient was the same. Predicted amino acid sequence in this study was the

same as MAGE-1 amino acid sequence from skin tissue of melanoma patient (M77481 and NM_004988) and there was one amino acid difference from melanoma skin tissue (BC017555) and liver tissue of hepatocellular carcinoma patient (AY148486), the T32A.

CONCLUSIONS

MAGE-1 gene coding area can be isolated from the result of FNAB from liver tissue of hepatocellular carcinoma patients and normal testicular tissue using nested PCR technique with final outcome a band of + 931 pb, containing 927 nucleotides that encode 309 amino acids. MAGE-1 gene coding area from liver tissue of hepatocellular carcinoma patient and normal testicular tissue had 100% homology with sequence accession number M77481 (sequence from melanoma skin tissue), NM_004988 (sequence from melanoma skin tissue) and 99% with s accession number BC017555 (sequence from melanoma skin tissue) and AY148486 (from liver tissue of hepatocellular carcinoma patients).

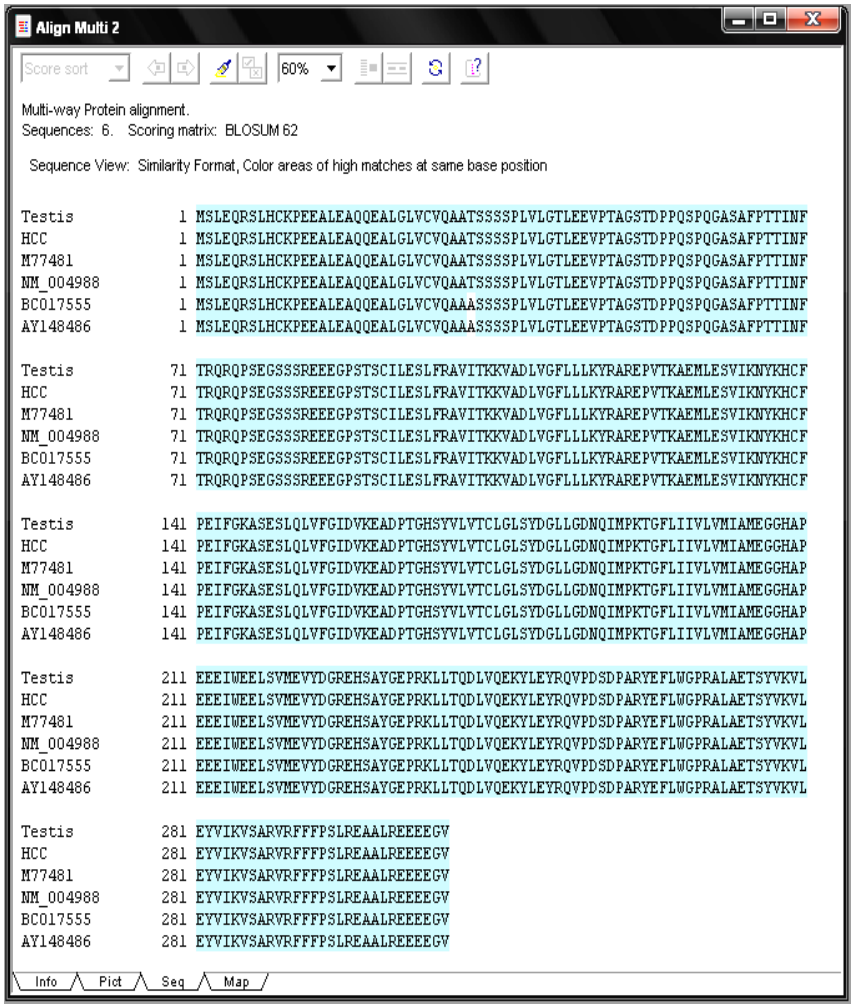

Figure 6. Result of analysis of MAGE-1 amino acid prediction fron normal testicular tissue and liver tissue of carcinoma patients with MAGE-1 amino acid in GeneBank.

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