Full Length Research Paper

Relationship between H-RAS oncogene and uterine tumor

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The current study is skillful to detect the mutations of the H-RAS oncogene in patient of uterine cancer. Thirty specimens of blood and tissue were collected for DNA extraction, H-RAS oncogene amplification and histopathology examination. The results, which revealed presence of mutations in H-RAS oncogene represented by homozygous wild type Leu/Leu was 20%, mutant translocation genotype Val/Val was 48%, mutant deletion genotype Val/Leu was 22% and heterozygous genotype Leu /Val was 10%. The histopathological results of this study reveal too many types of uterine cancer. The major types of uterine cancer that appear in this study represented by endometrial adenosquamous carcinoma are associated with endometrial leiomyosarcoma, the other cases were represented as endometrial spheroidal cell carcinoma and as endometrial intra epithelial carcinoma.

Key words: H-RAS gene, uterine cancer, DNA.

INTRODUCTION

Uterine cancer begins when normal cells in the uterus change and grows uncontrollably, forming a mass called a tumor. There are two major types of uterine cancer, adenocarcinoma, and this type of cancer makes up more than 95% of uterine cancers; it develops from cells in the lining of the endometrium. This cancer is also commonly called endometrial cancer. Sarcoma is the second form of uterine cancer that develops in the myometrium or in the supporting tissues of the uterine glands (Cancer_Net .mht, 2011). It has been reported that the ras gene family (K-RAS, H-RAS, and N-RAS) is associated with the development of human neoplasms (Barbacid, 1987). Single-point mutations of the ras gene, usually at codons 12, 13, and 61, result in a single amino acid substitution in critical domains, and this substitution has a significant role in tumor development by making the proteins no longer dependent on GTPase (guanosine5’-triphosphatase)-activating protein regulation. Frequent ras mutations have been reported in a number of human cancers, including adenocarcinoma of the pancreas (90%), colon (50%), thyroid (50%), and lung (30%) (Bos, 1989). The H-RAS gene commonly is activated in human urinary tract tumors. H-RAS mutations have been reported in malignant fibrous histiocytoma (MFH), leiomyosarcoma, and rhabdomyosarcoma as for sarcomas (Wilke et al., 1993; Bohle et al., 1996; Yoo and Robinson, 1999; Yoo et al., 1999).

MATERIALS AND METHODS

A total of thirty patients women with uterine cancer were contacted after surgery. The blood samples were collected in sterilized tube with EDTA, brought to the laboratory and kept directly in -20°C till used for DNA extraction, while the tissue samples were collected from endometrial cancer for histopathological study and kept in formalin 10% for 48 h (Luna,1968). Genomic DNA was isolated by DNA extraction kit. The mutation of the codon 12 of H-ras oncogene was studied according to protocol of Chikako et al. (1994). Briefly genomic DNA was amplified using the primers Forward5´-CTCTATAGGG ATCATAC-3´, Reverse5´-GACTCCTACCGGA AAC AGG-3´. PCR reaction mix and condition are PCR green master mix 12.5 µl, Primer forward 1 µl, Primer reverse1 µl, DNA 5 µl, D.W. 5.5 and 25 µl mineral oil. PCR conditions were denaturation 94°C for 5 min 1 cycle, denaturation 94°C for 1 min annealing 58°C for 1.5 min, extension 72°C for 2.5 min. and 30 cycles and extension 72°C for 5 min 1 cycle. The PCR product was 108 pb, and then subjected to electrophoresis on a 2% agarose gel. PCR product was digested for 3 h at 37°C with Eco R1 restriction enzyme using 3 µl of NE buffer 2, 0.5 µl (5 units) of enzyme and 10µl of PCR product, 0.3 µl BSA. The PCR products were classified as homozygous wild type (80 to 28 bp), translocation mutant genotype (108 bp), heterozygous (108-80 bp) alleles, deletion mutant genotype (80 bp).
RESULTS AND DISCUSSION

The results showed the mutations of the H-RAS oncogene (Figure 1). The frequency of patient with uterine cancer H-RAS oncogene homozygous wild type Leu/Leu was 20%, while that of mutant translocation genotype Val/Val was 48%. The mutant deletion genotype Val/Leu was 22%. The heterozygous genotype Leu/Val was 10%. The histopathological results of the current study revealed too many types of uterine cancer (Table 1). Twenty six cases from the total were represented by endometrial adenosquamous carcinoma associated with 16 cases endometrial leiomyosarcoma (Figures 2, 3, 5, 6 and 9), three cases were represented as endometrial spheroidal cell carcinoma (Figures 4 and 7). One case appears as endometrial intra epithelial carcinoma (Figure 8).

CONCLUSIONS AND RECOMMENDATIONS

Increased risk of uterine cancer incidence in women who had mutant translocation genotype deletion and heterozygous genotype of H-RAS oncogene, as most of...
Table 1. Show the type and percentage of uterine cancer.

<table>
<thead>
<tr>
<th>Type of uterine cancer</th>
<th>Number of cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial adenosquamous carcinoma with</td>
<td>26</td>
<td>86.667</td>
</tr>
<tr>
<td>Endometrial lieomyosarcoma (16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrial spheriodal cell carcinoma</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Endometrial intra epithelial carcinoma</td>
<td>1</td>
<td>3.333</td>
</tr>
</tbody>
</table>

* 16 cases of endometrial lieomyosarcoma associated with the 26 cases of endometrial adenosquamous carcinoma.

Figure 2. (H and E 250X).
Figure 3. (H and E 250X).
Figure 5. (H and E280X).
the cases, were endometrial adenocarcinomas with leiomysarcoma. This study recommended that women should be less exposed to radiation, chemical hazardous substances and treatment with hormones to reduce mutation. Further studies are required to determine the relationship between endometrial cancer and other related genes.
REFERENCES


