K-ras gene point mutations in human endometrial carcinomas: correlation with clinicopathological features and patients’ outcome

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Abstract In order to evaluate the role of K-ras gene point mutations in the progression of endometrial carcinoma, we applied the polymerase chain reaction/restriction-fragment-length polymorphism technique to 57 tumours surgically removed from women of Polish origin. We assessed the relationship between K-ras gene activation and clinicopathological features as well as patients’ outcome. Mutational activation in codon 12 of the K-ras gene was detected in 8 out of 57 (14%) endometrial carcinomas, while in codon 13 of the K-ras gene no point mutations were noted. A correlation between the histological type of the tumour and codon 12 K-ras gene mutation was noted (\( P < 0.05 \); Fisher exact test). K-ras gene mutation was not related to the patients’ age, surgical stage, histological grade or to the depth of myometrial invasion. A trend towards a poorer prognosis was noted during the follow-up of patients whose tumours had shown K-ras codon 12 point mutations, but the difference was not significant (\( P = 0.06 \); log-rank test). Our data indicate that point mutations in codon 12 of the K-ras gene are a rare event in human endometrial carcinomas. The lack of correlation between K-ras point mutations and clinicopathological features (except histological type) supports the hypothesis of a random activation of the K-ras gene in human neoplastic endometrium.

Key words K-ras · Endometrial cancer · Patients’ outcome · PCR-RFLP

Abbreviations PCR polymerase chain reaction · RFLP restriction-fragment-length polymorphism · FIGO International Federation of Obstetrics and Gynaecology · WHO World Health Organisation

Introduction

In recent years considerable progress has been made in our understanding of the molecular basis of carcinogenesis in humans. Data on the development of human neoplasia include the activation of protooncogenes as well as the inactivation of the tumour-suppressor genes (Bishop 1991; Vogelstein and Fearon 1993).

More than 200 human oncogenes have been discovered and cloned (Hesketh 1997). One of the gene families in which mutational activation has been widely detected in various human neoplasms is the ras gene family (Bos 1989; Rodenhuis 1992; Waldmann and Rabes 1996). N-ras gene point mutations are frequently noted in myeloid leukemias (Bos et al. 1985; Janssen et al. 1987). H-ras alterations are detected mainly in cervical and bladder
carcinomas (Riou et al. 1988; Lee et al. 1996). Point mutations of the K-ras gene are frequently reported in adenocarcinomas of the pancreas, colon, lung and ovary (Urban et al. 1993; Bos et al. 1987; Li et al. 1994; Rodenhuis and Slebos 1992; Enomoto et al. 1991a).

The incidence of first-exon K-ras gene point mutations ranges from 10% to 30% in human endometrial carcinomas (Jeyarajah et al. 1996). However, it is worth noting that the higher incidence of K-ras mutations was observed among samples obtained from Japanese women rather than women from the USA (Enomoto et al. 1995; Sasaki et al. 1993).

In our previous study, the expression of the ras p21 protein was immunohistochemically identified in 75% of the human endometrial carcinomas analysed, and a correlation between p21 protein immunostaining and the depth of myometrial invasion has also been noted (Semczuk et al. 1997a). We have identified point mutations at the first exon of the K-ras gene in 2 out of 13 (15%) human endometrial carcinomas and in a single case of ovarian metastasis to the endometrium (Semczuk et al. 1997b). As a preliminary result, 2 out of 17 (14%) of the endometrial neoplasms (both endometrial carcinomas of the endometrioid type) were positive for codon 12 mutations of the K-ras gene as determined by the polymerase chain reaction/restriction-fragment length polymorphism (PCR-RFLP) method (Semczuk et al. 1997c). However, to the best of our knowledge, mutational activation of the K-ras gene has not been studied in a large group of endometrial cancer patients from Eastern Europe.

Therefore, the aim of this study was to assess the clinicopathological features and prognostic utility of point mutations in codons 12 and 13 of the K-ras gene in human endometrial carcinomas obtained from women of Polish origin.

### Patients and methods

#### Patients

A group of 57 patients with endometrial cancer, who underwent a total abdominal hysterectomy/bilateral salpingo-oophorectomy at the 2nd Department of Gynaecological Surgery, Lublin School of Medicine, Poland, between 1993 and 1997, were included in the study. The average age of the patients was 62 years (range 47–81 years). None of the patients had received chemotherapy, hormonal therapy, or radiation therapy prior to surgery.

The clinical stage of the disease was classified according to the revised FIGO criteria (FIGO 1989). The majority of patients (46) had tumours at stage I, 2 patients had tumours classified at stage II, 7 patients were classified at stage III, and 2 patients were classified at stage IV. The samples were grouped by histological grade (according to FIGO): 17 neoplasms were G1, 27 were G2 and 13 were G3.

Histological features were classified according to WHO (Scully et al. 1994). The tumours were classified as follows: 40 cases were endometrioid endometrial carcinomas, 14 were adenosquamous carcinomas and 3 were clear-cell carcinomas. There was no myometrial invasion of the neoplasm in 15 cases, invasion of less than half of the myometrial wall was found in 20 cases and in 22 cases there was invasion involving more than half of the myometrial wall.

#### DNA isolation

A portion of the tissue was fixed in buffered formalin (pH 7.4) for a routine histopathological examination, while the remainder was immediately frozen in liquid nitrogen and stored at −80°C. DNA was isolated by the standard proteinase K/phenol/chloroform procedure (Sambrook et al. 1989).

#### PCR

PCR was performed with 0.5 ng DNA in a final volume of 50 μl containing 200 μmol/l each dNTP, 1.75 mM MgCl₂, and a 10× Taq polymerase buffer. The primers used in the study were as follows (Jiang et al. 1989):

K5’ 5’ACCTGAATATAACCTTGATGTTGACCT3’
K3’ 5’CTAAAGAATGGTCCTGGACC3’

After the initial 5 min denaturation step at 94°C had been performed, 5 U Taq polymerase DNA (MBI Fermentas, Lithuania) was added (the “hot-start” PCR technique; Rolfs et al. 1992). The samples were subjected to 25 cycles of amplification under the following conditions: denaturation for 30 s at 94°C, annealing for 40 s at 54°C and extension for 30 s at 72°C. A 10-μl sample of each PCR product was visualized by electrophoresis on a 10% native polyacrylamide gel to confirm the PCR reaction.

#### RFLP

We used the RFLP method for detecting the point mutations of codons 12 and 13 of the K-ras gene according to the procedure previously described (Jiang et al. 1989). PCR amplification primers revealed a 157-nucleotide fragment of the first exon of the K-ras gene. After incubation with endonuclease BstNI (Sigma-Aldrich, Germany), the wild-type K-ras gene fragment was cut enzymically into three fragments (114, 29 and 14 nucleotides respectively). When a point mutation at either the first or second position of codon 12 of the K-ras occurred, BstNI would cleave once (143 bp and 14 bp). In order to identify the point mutations of the K-ras gene, we applied 30 U BstNI to 10 μl PCR-amplified product using a commercial buffer (Palette Buffer Blue; Sigma-Aldrich, Germany) in a total volume of 50 μl. The samples were digested for 3 h at 60°C. Samples comprising 20 μl digested PCR products were electrophoresed on a 10% native polyacrylamide gel. Afterwards, each gel was stained with ethidium bromide and photographed on an ultraviolet light transilluminator.

K-ras codon 13 mutations were detected by digestion of the 157-nucleotide fragment by restriction endonuclease HphI (Sigma-Aldrich, Germany). The substitution of glycine for aspartic acid created the sequence GTTGA, which is recognized by the endonuclease, which cut the 157-nucleotide fragment into bands of 114 and 43 nucleotides. A 10-μl sample of the amplified PCR product was digested (3 h at 37°C) by 8 U HphI in a buffer (Palette Buffer Green; Sigma-Aldrich, Germany) containing the enzyme in a total volume of 50 μl. The digested fragments were visualized by electrophoresis on a 10% native polyacrylamide gel, stained with ethidium bromide and photographed.

Human placenta and endometrial carcinomas with mutations in the K-ras gene (Semczuk et al. 1997b) were used as negative and positive controls respectively.

#### Statistical analysis

Statistical analysis of how the difference in the frequency of K-ras point mutations varied with age and clinicopathological features was carried out using Fisher’s exact test, the $\chi^2$-test, and Student’s $t$-test. A cumulative proportional survival rate was calculated by the Kaplan-Meier method (1958), and the data were statistically analysed by the log-rank test. Differences were considered significant if the $P$ value was less than 0.05.