Dection of mutations in cancer-related genes using Muts mismatch binging protein and its clinical application to diagnosis of digestive cancers.

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Dection of mutations in cancer-related genes using Muts mismatch binging protein and its clinical application to diagnosis of digestive cancers.

Research Project

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11670486

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Section

一般

Research Field

Gastroenterology

Research Institution

Kanazawa University

Principal Investigator

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MutS protein / homoduplex / heteroduplex / Cancer cell lines / pancreatic juice / K-ras mutation / p53 mutation

Research Abstract

Recently, highly sensitive method of detecting genetic abnormalities including all single base mutations, as well as small deletions or insertions at any locations using Mut S mismatch binding protein was demonstrated in the experimental reports of Lishanski et al. and Wagner et al. I tried to apply this new method to detection of changes of cancer-related genes. First, DNA was extracted from 4 pancreatic cancer cell lines and pancreatic juice (PJ) from 5 patients with pancreatic carcinoma (PCa) and 10 with chronic pancreatitis (CP). Biotin-labeled sense primers for K-ras codon 12, exon 5 to 8 of p53, and exon 1 to 3 of p16 were synthesized. After the process of touchdown PCR amplification, heat denaturation and re-annealing, heteroduplex PCR products form in the case of presence of mispaired and unpaired bases and otherwise homoduplex PCR products alone form. Because of the specific binding of the heteroduplex not homoduplex to immobilized MutS protein on the nitrocellulose membrane, chemiluminescence method using streptavidin-HRP was performed for the detection of heteroduplex which is specific binding to MutS protein.
Among 4 pancreatic cancer cell lines, that is, PANC-1, PaCa-2, BxPC-3, and HPAF, PaCa-2 (homozygous mutation of K-ras codont 12) and BxPC-3 (no mutation of K-ras codon 12) would theoretically form homoduplex alone. PANC-1 and HPAF would also form heteroduplex due to heterozygous mutation of K-ras codon 12. On the other hand, p53 mutations were already detected in PANC-1 (exon 8), PaCa-2 (exon 7), BxPC-3 (exon 6), HPAF (exon 5) by direct sequencing. Therefore, as the same manner, these p53 mutations theoretically form heteroduplex. However, false positive was recognized in PaCa-2 for K-ras exon 1 and in PANC-1 for p53 exon 5, PaCa-2 for p53

exon 6, and BxPC-3 for p53 exon 7.Moreover, although the incidence of this heteroduplex using MutS assay was 80% (4/5) for K-ras exon 1 and 60% (3/5) for p53 in PJ with PCa, it was also 60% (6/10) for K-ras exon 1 and 50% (5/10) for p53 in PJ with CP.This detecting method has high sensitivity, but the problem of frequent false positivity.
The cause of the false positivity was regarded as the misincorporation during PCR and imperfect removal of non-specific biotion from the nitrocellulose membrane after the reaction with immobilized MutS binding protein. Further improvement will be needed for this detecting method.▲ Less

Research Products (16 results)

All Other

All Publications

[Publications] Ha,A.,Watanabe,H., et al.: "Usefulness of supernatant of pancreatic juice for genetic analysis of K-ras in diagnosis of pancreatic carcinoms."Pancreas. (in press). (2001)

[Publications] Hu,YX.,Watanabe,H., et al.: "An immunohistochemical analysis of p27 expression in human pancreatic carcinomas."Pancreas. 21巻3号. 226-230 (2000)

[Publications] Watanabe,H.,Ha,A., et al.: "K-ras mutations in duodenal aspirate without secretin stimulation for screening of pancreatic and biliary tract carcinoma"Cancer. 86巻8号. 1441-1448 (1999)

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