Expression of pituitary tumor transforming gene in human gastric carcinoma

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Abstract

AIM: Pituitary tumor transforming gene (PTTG1) is overexpressed in a variety of tumors, including carcinomas of the lung, breast, colon, as well as in leukemia, lymphoma and pituitary adenomas. However, there is little information on its expression in gastric carcinoma. We sought to investigate the expression of PTTG1 in gastric carcinoma and to explore the relationship between its expression and clinicopathological factors.

METHODS: We studied 75 primary human gastric adenocarcinomas, including 17 mucosal carcinomas, 21 submucosal infiltrative carcinomas, 12 carcinomas invading proprial muscle layers, 6 carcinomas reaching the subserosa, and 19 carcinomas penetrating the serosal surface. Immunohistochemical analysis was performed using paraffin-embedded sections of gastric adenocarcinomas.

RESULTS: PTTG1 was expressed heterogeneously in carcinomas. Positive PTTG1 staining was observed in 65.3% of the carcinomas (49 of 75). Its expression did not correlate significantly with either the histological type or the depth of infiltration of the gastric carcinomas. However, a statistical analysis showed significant differences between the primary adenocarcinomas and the associated metastatic lymph nodes.

CONCLUSION: The results of this study demonstrate that PTTG1 expression is enhanced in metastatic lymph nodes in comparison to that in primary carcinomas. We suggest that PTTG1 may contribute to lymph node metastases in gastric carcinoma.

INTRODUCTION

Gastric carcinoma is one of the most common causes of malignancy-related death worldwide. Recent molecular biological studies suggest that genetic instability may play an important role in the pathogenesis of gastric carcinogenesis[6]. There are at least two distinct genetic instabilities in gastric tumorigenesis. One is chromosomal instability and the other is instability of the microsatellites. In the former, diminished expression of tumor suppressor genes, such as p53, Rb, APC, MCC and DCC, plays an important role in carcinogenesis. Whereas in the latter, the defective repair of mismatched bases results in an increase in the rate of point mutations[2-4].

Aneuploidy is a numerical imbalance in chromosomes caused by missegregation during cell division. A critical event that promotes equal partitioning of chromosomes during mitosis is the proper and timely separation of sister chromatids attached to each other and to the mitotic spindle. Interestingly, pituitary tumor transforming gene (PTTG1) is a securin that acts as an inhibitor of chromatid separation[5].

PTTG1 is a novel onco gene that has been identified by Pei et al.[6]. PTTG1 overexpression in mouse fibroblasts and NIH 3T3 cells induced cellular proliferation and transformation, both in vitro and in vivo[7,8]. PTTG1 is overexpressed in a variety of tumors, including carcinomas of the lung, breast, colon, as well as in leukemia, lymphoma and pituitary adenomas[8,9,11]. The expression of PTTG1 in normal tissues is restricted with the highest expression occurring in the testis[6,8,9]. PTTG1 is expressed in a stage-specific manner in germ cells during the spermatogenic cycle, suggesting that it may play a role in male germ cell differentiation[10]. PTTG1 also regulates the secretion of basic fibroblast growth factor[4].

In the present study, we investigated PTTG1 expression and explored the relationship between its expression and clinicopathological factors in 75 gastric carcinoma specimens.

MATERIALS AND METHODS

We studied 75 primary human gastric adenocarcinomas, including 17 mucosal carcinomas, 21 submucosal infiltrative carcinomas, 12 carcinomas invading proprial muscle layers, 6 carcinomas reaching the subserosa, and 19 carcinomas penetrating the serosal surface. All tumor specimens were obtained from patients at the Nagasaki University Hospital between 2001 and 2002. Each tumor was assigned a histological type and a depth grading of infiltration according to the
Japanese Classification of Gastric Carcinoma by the Japanese Gastric Cancer Association[13]. The primary human gastric adenocarcinomas were classified histologically as follows: 4 papillary adenocarcinomas, 18 tubular adenocarcinomas of the well differentiated type, 22 tubular adenocarcinomas of the moderately differentiated type, 7 poorly differentiated adenocarcinomas of the solid type, 10 poorly differentiated adenocarcinomas of the non-solid type, 12 signet-ring cell carcinomas, and 2 mucinous adenocarcinomas. Diagnosis was established by two independent pathologists (CY Wen and T Nakayama) and cases of questionable diagnosis were omitted from the study.

Immunohistochemistry
Formalin-fixed and paraffin-embedded tissues were cut into 4 µm sections, deparaffinized in xylene, and rehydrated in phosphate-buffered saline. Deparaffinized sections were subsequently preincubated in 3% H2O2 for 30 min, followed by incubation in normal bovine serum to prevent nonspecific binding and subsequently incubated overnight at 4°C in a primary polyclonal antibody directed against human PTTG1 (2 µg/ml) (Zymed Laboratories, Inc. South San Francisco, CA, USA). Next, the slides were incubated in biotinylated anti-rabbit immunoglobulin G followed by avidin-horseradish peroxidase and the reaction product was resolved using diaminobenzidine (DAB) (Vectastain ABC kit; Vector Laboratories, Burlingame, CA, USA). For the PTTG1 expression, pituitary adenoma served as a positive control, and the slide with the primary antibody omitted was used as a negative control. Analysis of the immunohistochemical staining was performed by two investigators (CY Wen and T Nakayama). PTTG1 expression was classified into three categories depending on the percentage of cells stained and/or the intensity of staining: -, 0% to 10% positive tumor cells; +, 10% to 50% positive tumor cells; and ++, >50% positive tumor cells.

Statistical analysis
Statistical analyses were performed using Spearman’s correlation coefficient by rank test and the Mann-Whitney U test. A P value <0.05 was accepted as statistically significant.

RESULTS
PTTG1 protein was detected in the cytoplasm. Benign gastric epithelia showed focal and patchy immunoreactivity of PTTG1 with faint to mild staining intensity. The results of immunohistochemical analysis are summarized in Table 1. PTTG1 was expressed heterogeneously in carcinomas. Positive PTTG1 staining was observed in 65.3% of carcinomas (in 49 out of 75). The correlation between PTTG1 immunoreactivity and tumor histological type is shown in Table 1. Tubular adenocarcinomas of the well and moderately differentiated types were stained strongly for PTTG1 (Figure 1). However, poorly differentiated adenocarcinomas of the solid and non-solid types exhibited weak PTTG1 expression (Figure 2) and mucinous adenocarcinomas were negative for antibody reaction.

Table 1 Histological types of gastric adenocarcinomas and immunohistochemical PTTG1 staining (75 cases)  n(%)

|                      | n | - | + | ++ | P value |
|----------------------|---|---|---|----|--------|
| Total carcinomas     | 75| 26(34.7) | 31(41.3) | 18(24.0) |
| Papillary adenocarcinomas | 4 | 1(25.0) | 2(50.0) | 1(25.0) |
| Tubular adenocarcinomas | 42 | 18(33.3) | 6(33.3) | 6(33.3) |
| Well differentiated   | 18 | 6(33.3) | 6(33.3) | 6(33.3) |
| Moderately differentiated | 22 | 7(31.8) | 7(31.8) | 8(36.4) |
| Poorly differentiated adenocarcinomas | 42 | 7(16.7) | 6(14.3) | 7(16.7) |
| Solid types           | 7 | 4(40.0) | 2(28.6) | 1(14.3) |
| Non-solid type        | 10 | 4(40.0) | 6(60.0) | 0(0.0) |
| Signet-ring cell carcinoma | 12 | 4(33.3) | 7(58.3) | 1(8.3) |
| Mucinous adenocarcinoma | 2  | 2(100.0) | 0(0.0) | 0(0.0) |

See Materials and Methods for classification of staining intensity.
between primary adenocarcinomas and metastatic lymph nodes (P<0.005, Table 3).

**Table 2** Invasive grades of gastric adenocarcinomas and PTTG1 immunohistochemistry (75 cases) (n,%)

| Grade | - | + | ++ | P value |
|-------|---|---|----|--------|
| m     | 17 | 6(35.3) | 5(29.4) | 6(35.3) |
| sm    | 21 | 5(23.8) | 11(52.4) | 5(23.8) |
| mp    | 12 | 4(33.3) | 3(25.0) |
| ss    | 6  | 2(33.3) | 4(66.7) | 0(0.0) |
| se    | 19 | 8(42.1) | 7(36.8) | 4(21.1) |
| Total | 75 | 26(34.7) | 31(41.3) | 18(24.0) |

m, mucosal carcinomas; sm, submucosal infiltrative carcinomas; mp, carcinomas invading the proprial muscle layers; ss, carcinomas reaching the subserosa; se, carcinomas penetrating the serosal surface.

**Table 3** PTTG1 expression in primary adenocarcinomas and metastatic lymph nodes (19 cases) (n,%)

|          | n | - | + | ++ | P value |
|----------|---|---|---|----|--------|
| Primary adenocarcinomas | 19 | 12(63.2) | 7(36.8) | 0(0.0) |
| Metastatic lymph nodes  | 19 | 4(21.0) | 9(47.2) | 6(31.6) |

In conclusion, the results of this study demonstrate that PTTG1 expression is enhanced in metastatic lymph nodes in comparison to that in primary carcinomas. We suggest that PTTG1 is a marker of lymph node metastasis in gastric carcinomas.

**REFERENCES**

1. Fang DC, Jess JR, Wang DX, Zhou XD, Luo YH, Young J. Infrequent loss of heterozygosity of APC/MCC and DCC genes in gastric cancer showing DNA microsatellite instability. J Clin Pathol 1999; 52: 504-508
2. Fang DC, Yang SM, Zhou XD, Wang DX, Luo YH. Telomere erosion is independent of microsatellite instability but related to loss of heterozygosity in gastric cancer. World J Gastroenterol 2001; 7: 522-526
3. Martins C, Kedda MA, Kew MC. Characterization of six tumor suppressor genes and microsatellite instability in hepatocellular carcinoma in southern African blacks. World J Gastroenterol 1999; 5: 470-476
4. Wu BP, Zhang YL, Zhou DY, Gao CF, Lai ZS. Microsatellite instability, MMR gene expression and proliferation kinetics in colorectal cancer with familial predisposition. World J Gastroenterol 2000; 6: 902-905
5. Zou H, McGarry TJ, Bernal T, Kirscher MW. Identification of a vertebrate sister-chromatid separation inhibitor involved in translocation and tumorigenesis. Science 1999; 285: 438-422
6. Pei L, Melmed S. Isolation and characterization of a pituitary tumor-transforming gene. M d Endocrinol 1997; 11: 433-441
7. Kakar SS, Jennes L. Molecular cloning and characterization of the tumor transforming gene (TUTR 1): A novel gene in human tumorigenesis. Cytogenet Cell Genet 1999; 84: 211-216
8. Zhang H, Horwitz GA, Precant TR, Valentini A, Nakashima M, Bronstein MD, Melmed S. Structure, expression and function of a human pituitary transforming gene (PTTG). M d Endocrinol 1999; 13: 156-166
9. Dominguez A, Ramos-Morales F, Romero F, Rios RM, Dreyfus F, Tortolero M, Pintor-Toro JA. Ptttg, a human homologue of rat pttg, is overexpressed in hematopoietic neoplasms. Evidence for a transcriptional activation function of hPTTG. Oncogene 1998; 17: 2187-2193
10. Heaney AP, Singson R, McCabe CJ, Nelson V, Nakashima M, Melmed S. Expression of pituitary-transforming gene in colorectal tumors. Lancet 2000; 355: 716-719
11. Saez C, Japan MA, Ramos-Morales F, Romero F, Segura DI, Tortolero M, Pintor-Toro JA. Ptttpg is over-expressed in pituitary adenomas and other primary epithelial neoplasias. Oncogene 1999; 18: 5473-5476
12. Pei L. Genomic organization and identification of an enhancer element containing binding sites for multiple proteins in rat pituitary tumor-transforming gene. J Bid Chen 1999; 273: 5219-5225
13. Japanese Gastric Cancer Association. Japanese Classification of Gastric Carcinoma (The 13th Edition). Edited by Japanese Gastric Cancer Association, Tokyo, Kenheira Press 1999
14. Loeb LA. Mutator phenotype may be required for multistage carcinogenesis. Cancer Res 1991; 51: 3075-3079
15. Hartwell L. Defects in a cell cycle checkpoint may be responsible for the genomic instability of cancer cells. Cell 1992; 71: 543-546
16. Furuya T, Uchiyama T, Murakami T, Adachi A, Kawauchi S, Oga A, Hirano T, Sasaki K. Relationship between chromosomal instability and intratumoral regional DNA ploidy heterogeneity in primary gastric cancers. Clin Cancer Res 2000; 6: 2815-2820
17. Choma D, Daures JP, Quantin X, Pujol JL. Aneuploidy and prognosis of non-small-cell lung cancer: a meta-analysis of published data. Br J Cancer 2003; 88: 14-22
18. Lengauer C, Kinzler KW, Vogelstein B. Genetic instability in human cancers. Nature 1998; 396: 643-649
19. Shibata Y, Haruki N, Kukwabara Y, Nishikawa T, Kato J, Shinoda N, Sato A, Kimura M, Koyama H, Toyama T, Ishiguro K, Kudo J, Tarashita Y, Konishi S, Fuji Y. Expression of PTTG (Pituitary Tumor Transforming Gene) in esophageal cancer. Jpn J Clin Oncol 2002; 32: 233-237
20. Pei L. Identification of c-myc as a down-stream target for pituitary tumor-transforming gene. J Biol Chem 2001; 276: 8484-8491
21. Zetter BR. Angiogenesis and tumor metastasis. Annu Rev Med 1998; 49: 407-424

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