

# High Level of Expression of $\alpha$ -Fetoprotein Receptor in Gastric Cancers

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## Key Words

$\alpha$ -Fetoprotein ·  $\alpha$ -Fetoprotein receptor · Gastric cancer · Receptors · Tumor markers

## Abstract

The expression of the receptor for  $\alpha$ -fetoprotein (AFP-R) was examined immunohistochemically in 47 cancer and 14 benign human gastric tissues. Rabbit polyclonal antibody against human AFP-R was used for immunohistochemical staining. Thirty-four of the 47 cancer tissues expressed AFP-R showing granular or reticular staining on the cancer cell surface, while only 2 of 61 control cases (14 benign gastric tissues and 47 nonmalignant tissues adjacent to cancer) showed faint and homogeneous staining in the cytoplasm of noncancerous cells. There was a significant difference in staining intensity between the cancerous and noncancerous groups. However, no statistically significant difference in staining intensity was found among the groups of well-differentiated, moderately differentiated and poorly differentiated adenocarcinomas. On the other hand, the staining intensity of signet ring cell carcinoma was significantly weaker than that of the three adenocarcinoma groups. The

high level of AFP-R expression in gastric cancers may allow the use of AFP-R as a new clinically useful marker of gastric cancer in the tissue level.

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## Introduction

$\alpha$ -Fetoprotein (AFP) was reported as the first oncofetal antigen in 1963 [1]. The serum concentration of AFP decreases to an almost undetectable level postnatally, and it becomes markedly elevated in patients with hepatocellular carcinoma [2], yolk sac tumor [3] and other types of malignancies, including gastric cancer [4, 5]. Therefore, AFP has been used as an important tumor marker in clinical diagnosis.

AFP has been shown to have various physiological functions, including regulation of cell proliferation and immune response, not only in normal embryonic and fetal tissues [6–9] but also in cancer tissues [5–8, 10–12]. AFP works as both up- and downregulators of cell growth depending on the conformational change of the tertiary structure [8, 12]. The transformation of AFP is induced by binding to some ligands, such as steroids or fatty acids.

During the courses of these studies, AFP was proven to be endocytosed by proliferating or differentiating cells via the receptor for AFP (AFP-R) on the cell surface [11–15]. Though the expression of AFP-R has been confirmed in several types of cells, there have been no studies on AFP-R expression in solid cancer tissues other than breast cancer [14]. The present study is the first to show a high level of AFP-R expression in gastric cancer cells in the tissue level.

## Materials and Methods

### Gastric Tissue Sections

Paraffin sections of 47 human gastric cancer tissues (13 well-differentiated, 21 moderately differentiated and 7 poorly differentiated adenocarcinoma tissues and 6 signet ring cell carcinoma tissues) and 14 benign gastric tissues (2 normal, 1 acute gastric ulcer, 2 chronic gastritis, 5 hyperplastic polyp and 4 adenoma tissues) were examined for the expression of AFP-R. Nonmalignant mucosal tissues adjacent to cancer were seen in all of the 47 cancer sections and were also examined as benign gastric tissues. All of these samples were randomly selected from our stock blocks of tissues resected surgically or endoscopically.

### Immunohistochemical Staining

The expression and localization of AFP-R in gastric tissues were examined by an immunohistochemical method. Rehydrated paraffin sections of 4- $\mu$ m thickness were stained using a Histo-RE-CAF kit (BioCurex, Richmond, Canada). The kit employs rabbit polyclonal antibody against human AFP-R purified from the extracts of MCF-7, a human breast cancer cell line, by affinity chromatography on an immobilized AFP column. The sections were treated with 3% hydrogen peroxide in methanol to minimize endogenous peroxidase activity, blocked with normal goat serum and reacted with rabbit anti-human AFP-R serum followed by horse-radish-peroxidase-conjugated goat anti-rabbit secondary antibody (DakoCytomation, Glostrup, Denmark). The treated sections were stained with a mixture of 3'-3-diaminobenzidine tetrahydrochloride and hydrogen peroxide. Matched slides were stained with the standard hematoxylin-eosin. The stained sections were mounted with Permafluor (Immunotech, Marseille, France) and examined with a Zeiss Axiophot microscope (Carl Zeiss, Oberkochen, Germany).

### Grading of Staining Intensity

According to the most predominant findings, staining intensity of peroxidase reaction products was graded from 0 to 3: grade 0, negative staining; grade 1, weakly positive staining; grade 2, definitely positive staining, and grade 3, strongly positive staining. Weak or no staining of nonmalignant cells was taken as a reference for negative staining. The uniformity of staining intensity was monitored by simultaneous staining of serial sections of an AFP-R-positive colon cancer used as a staining control throughout this study.

### Statistical Analysis

Differences in staining intensity of AFP-R between the groups of gastric cancer and noncancerous tissues and among the different

**Table 1.** Expression of AFP-R in gastric tissues subdivided according to the histological malignancy

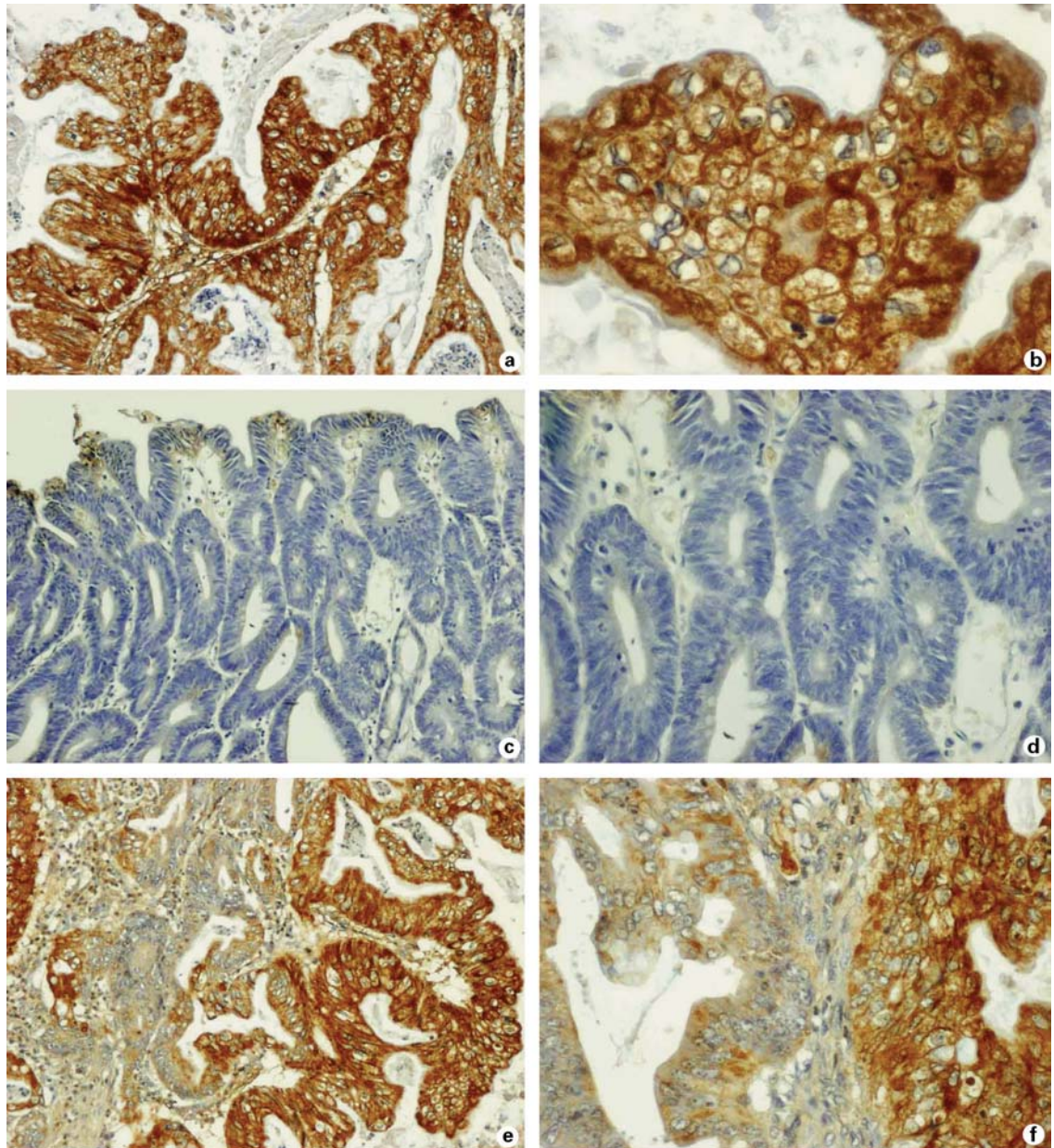
Tissue types	Samples	
	positive staining	total
<b>Gastric cancer</b>		
Well-differentiated	10	13
Moderately differentiated	17	21
Poorly differentiated	6	7
Signet ring cell	1	6
<b>Total</b>	<b>34</b>	<b>47</b>
<b>Benign</b>		
Noncancerous areas of the above patients	1	47
Normal	0	2
Acute gastric ulcer	0	1
Chronic gastritis	0	2
Hyperplastic polyps	0	5
Gastric adenoma	1	4
<b>Total</b>	<b>2</b>	<b>61</b>

histological types of gastric cancer were examined. Statistical analysis was performed with Stat View version 4.5 (Abacus Concepts, Berkeley, Calif., USA). The Mann-Whitney U test and the Kruskal-Wallis rank test were used to determine the significant differences. A difference was considered significant at  $p < 0.05$ .

## Results

### Expression of AFP-R in Gastric Tissues

In 34 of the 47 gastric cancer tissues, staining intensity for AFP-R varied in cancer cells (grade 0, 27.7%; grade 1, 10.6%; grade 2, 38.3%; and grade 3, 23.4%; table 1; fig. 1a, b). These cancer cells showed granular or reticular staining on the cell surface. On the other hand, nonmalignant mucosal cells adjacent to cancer or in the sections of benign gastric tissues showed faint and homogeneous staining in the cytoplasm in only 2 cases (table 1; fig. 1c, d). Even within the same cancer tissue, the staining was not necessarily homogeneous (fig. 1e, f). Some clusters of cancer cells were strongly positive, while others were weakly positive or even negative in some cases. In addition, when there were different histological types of cancer cells in the same section, there was no consistent difference in staining intensity with respect to the cell differentiation (pictures not shown). Similarly, staining intensity did not differ between the central parts and the invasive tumor margins.



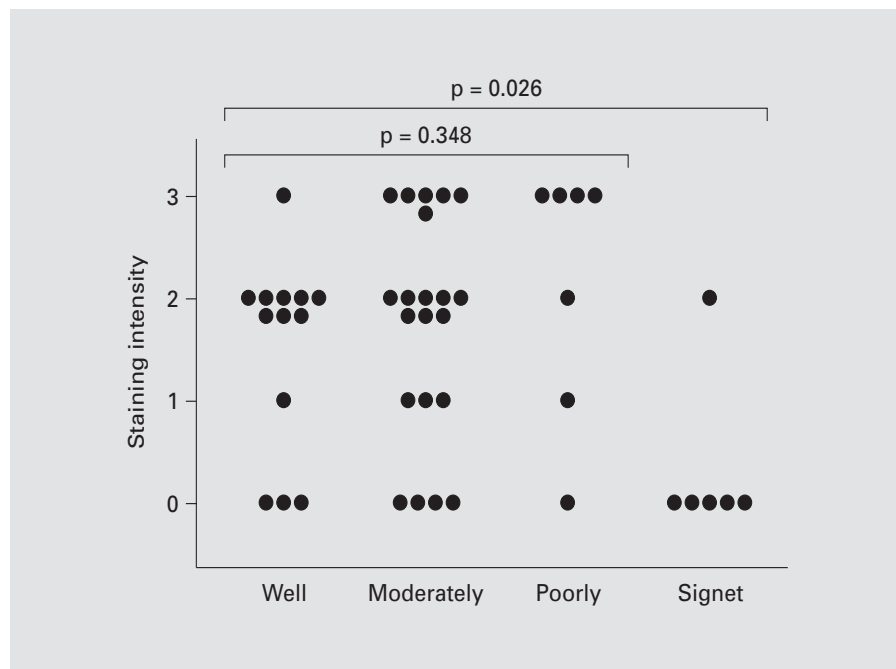
**Fig. 1.** Expression of AFP-R in gastric tissues. **a, b** Gastric cancer cells showed granular or reticular staining on the surface. **c, d** Gastric adenoma cells showed no staining except for 1 case, which showed faint and homogeneous staining (pictures not shown). **e, f** The staining intensity was heterogeneous even in the same gastric cancer section. **a, c, e**  $\times 100$ . **b**  $\times 400$ . **d, f**  $\times 200$ .

#### *Difference in Staining Intensity among Different Histological Types*

There was a statistically significant difference in staining intensity between the groups of gastric cancer and noncancerous tissues by the Mann-Whitney U test ( $p < 0.0001$ ). On the other hand, the Kruskal-Wallis rank test

showed no statistically significant difference among the groups of well-differentiated, moderately differentiated and poorly differentiated adenocarcinomas, while the staining intensity of signet ring cell carcinoma was significantly weaker than that of the other three groups ( $p < 0.05$ ; fig. 2).

**Fig. 2.** Statistical analysis of staining intensity among the different histological types of gastric cancer (well-, moderately and poorly differentiated cancers and signet ring cell carcinoma).



### Discussion

This study showed that in gastric cancer tissues AFP-R expression is increased compared with noncancerous gastric tissues. There was a significant difference between the staining intensities in the two groups ( $p < 0.0001$ ). In our unpublished observations, a considerable number of human colon cancer tissues were stained with the anti-AFP-R antibody used in this study as well as with labeled human AFP. Both methods showed identical patterns of cancer cell staining, and the staining with the labeled AFP was completely blocked with unlabeled purified AFP, suggesting that the AFP-R stained with the antibody represents an AFP-R.

Expression of AFP-R and uptake of AFP are regulated by the degree of cell differentiation in a variety of embryonic and fetal tissues [16, 17]. Since cancer cells share many common biochemical and antigenic features with embryonic and fetal cells, the cancer cells derived from tissues incorporating AFP during embryonic and fetal life would re-express the ability to take up AFP via AFP-R. Therefore, the expression of AFP-R in gastric cancer cells observed in this study suggests that embryonic and fetal gastrointestinal cells also express this receptor. Indeed, human embryonic and fetal gastrointestinal tracts produce AFP [18]. Thus, AFP might regulate the develop-

ment of the gastrointestinal tract by an AFP/AFP-R autocrine system.

Similarly, it was assumed that staining intensity representing the amount of AFP-R expression would also correlate with the degree of cancer cell differentiation. Unexpectedly, there was no statistically significant difference in staining intensity among the groups of well-differentiated, moderately differentiated and poorly differentiated adenocarcinomas. There are several possible explanations for the absence of a significant difference among these three groups. The staining was often heterogeneous even within the same cancer section. Cancer cells with strong staining tended to cluster together. These findings suggest that the expression of AFP-R is related to the cell cycle of cancer cells, or the nonuniformity of AFP-R staining may simply reflect the heterogeneous gene expression in malignant phenotypes, i.e. not only the undifferentiated expression but also the heterogeneous expression of phenotypes is characteristic of malignancy, as demonstrated in hepatocellular carcinoma tissues by Taketa et al. [19]. On the other hand, the staining intensity of signet ring cell carcinoma was significantly weaker. The lack of AFP-R expression in signet ring cell carcinoma may have a different significance in view of the specific cell structure filled with mucus in the cytoplasm.

The diverse characteristics of gastric cancer cells are another conceivable reason for the lack of a significant difference in staining intensity among the three adenocarcinoma groups. Gastric cancers have been phenotypically divided into two conventional groups, the intestinal and diffuse types described by Lauren [20] or the differentiated and undifferentiated types described by Sugano et al. [21]. However, recent developments in mucin histochemical and immunohistochemical techniques do not support this simple classification. The characteristics of gastric cancers are quite different not only histologically but also genetically, histogenetically and developmentally [22–25]. In many respects, the diversity of gastric cancers may be related to the various degrees of AFP-R expression.

Previous studies showed that AFP can work as a dual regulator, functioning in both up- and downregulation of cell proliferation [8, 12]. The steroid receptor superfamily induces conformational changes in the AFP molecule and exposes a hidden epitope capable of growth suppression [12]. On the other hand, the binding to unsaturated fatty acids, represented by arachidonic acid, transforms AFP into a variant being readily endocytosed as a growth promoter [12]. In this case, AFP works as a carrier of fatty acids essential for the growth of cancer cells via AFP-R. Newby et al. [15] demonstrated the presence of AFP-R on the cell surface in placental villous tissues, suggesting a possible receptor-mediated mechanism for AFP transport across the placenta between the fetal and maternal circulations. This finding also ascribes a role for AFP as

a carrier of some growth factors to proliferating cells. Thus, the biological role of AFP is too diverse and complicated to determine its general effect on the growth of cancer cells. However, it is clinically well known that AFP-producing gastric cancers have a higher malignant potential [4, 5]. It has also been shown that an anti-AFP antibody inhibits the growth of AFP-producing gastric cancer cells transplanted into nude mice [5]. Therefore, AFP would promote the growth of gastric cancer cells via AFP-R.

In this study, we showed a high level of AFP-R expression in gastric cancer, suggesting that AFP-R can be used as a new clinically useful marker of gastric cancer in the tissue level.

Further studies on the biological role of AFP in the regulation of cancer growth via AFP-R should lead to a novel cancer treatment [12]. On the other hand, Line et al. [26] showed the usefulness of Tc-99m AFP as a radiopharmaceutical to detect breast cancer. This method may be applicable to detect other kinds of cancers, including gastric cancer expressing AFP-R. The mechanisms of cell growth regulation by AFP/AFP-R remain to be elucidated.

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### References

- 1 Abelev GI, Perova SD, Khramkova NI, Postnikova ZA, Irlin IS: Production of embryonal  $\alpha$ -globulin by transplantable mouse hepatomas. *Transplantation* 1963;1:174–180.
- 2 Abelev GI: Alpha-fetoprotein in ontogenesis and its association with malignant tumors. *Adv Cancer Res* 1971;14:295–358.
- 3 Ballas M: Yolk sac carcinoma of the ovary with alpha fetoprotein in serum and ascitic fluid demonstrated by immunosmopphoresis. *Am J Clin Pathol* 1972;57:511–516.
- 4 Chen J, Rocken C, Treiber G, Jentsch-Ulrich K, Malfertheiner P, Ebert MP: Clinical implications of alpha-fetoprotein expression in gastric adenocarcinoma. *Dig Dis* 2003;21:357–362.
- 5 Takahashi Y, Ohta T, Mai M: Angiogenesis of AFP producing gastric carcinoma: correlation with frequent liver metastasis and its inhibition by anti-AFP antibody. *Oncol Rep* 2004;11:809–813.
- 6 Deutsch HF: Chemistry and biology of alpha-fetoprotein. *Adv Cancer Res* 1991;56:253–312.
- 7 Cavin LG, Venkatraman M, Factor VM, Kaur S, Schroeder I, Mercurio F, Beg AA, Thorgeirsson SS, Arsura M: Regulation of  $\alpha$ -fetoprotein by nuclear factor- $\kappa$ B protects hepatocytes from tumor necrosis factor- $\alpha$  cytotoxicity during fetal liver development and hepatic oncogenesis. *Cancer Res* 2004;64:7030–7038.
- 8 Dudich E, Semenkova L, Gorbatoeva E, Dudich I, Khromykh L, Tatulov E, Grechko G, Sukhikh G: Growth-regulative activity of human alpha-fetoprotein for different types of tumor and normal cells. *Tumor Biol* 1998;19:30–40.
- 9 Semeniuk DJ, Boismenu R, Tam J, Weissenhofer W, Murgita RA: Evidence that immunosuppression is an intrinsic property of the alpha-fetoprotein molecule. *Adv Exp Med Biol* 1995;383:255–269.
- 10 Li MS, Ma QL, Chen Q, Liu XH, Li PF, Du GG, Li G: Alpha-fetoprotein triggers hepatoma cells escaping from immune surveillance through altering the expression of Fas/FasL and tumor necrosis factor related apoptosis-inducing ligand and its receptor of lymphocytes and liver cancer cells. *World J Gastroenterol* 2005;11:2564–2569.
- 11 Li MS, Li PF, He SP, Du GG, Li G: The promoting molecular mechanism of alpha-fetoprotein on the growth of human hepatoma Bel7402 cell line. *World J Gastroenterol* 2002;8:469–475.
- 12 Mizejewski GJ: Biological role of  $\alpha$ -fetoprotein in cancer: prospects for anticancer therapy. *Expert Rev Anticancer Ther* 2002;2:709–735.
- 13 Suzuki Y, Zeng CQ, Alpert E: Isolation and partial characterization of a specific alpha-fetoprotein receptor on human monocytes. *J Clin Invest* 1992;90:1530–1536.

- 14 Moro R, Tamaoki T, Wegmann TG, Longenecker BM, Laderoute MP: Monoclonal antibodies directed against a widespread oncofetal antigen: the alpha-fetoprotein receptor. *Tumor Biol* 1993;14:116–130.
- 15 Newby D, Dalgliesh G, Lyall F, Aitken DA: Alphafetoprotein and alphafetoprotein receptor expression in the normal human placenta at term. *Placenta* 2005;26:190–200.
- 16 Moro R: Selective localization of alpha-fetoprotein and serum albumin within the sensory ganglia cells of developing chicken. *Neurosci Lett* 1983;41:253–257.
- 17 Uriel J, Trojan J, Moro R, Pineiro A: Intracellular uptake of  $\alpha$ -fetoprotein: a marker of neural differentiation. *Ann NY Acad Sci* 1983;417:321–329.
- 18 Gitlin D, Perricelli A, Gitlin GM: Synthesis of  $\alpha$ -fetoprotein by liver, yolk sac, and gastrointestinal tract of the human conceptus. *Cancer Res* 1972;32:979–982.
- 19 Taketa K, Shimamura J, Ueda M, Shimada Y, Kosaka K: Profiles of carbohydrate-metabolizing enzymes in human hepatocellular carcinomas and preneoplastic livers. *Cancer Res* 1988;48:467–474.
- 20 Lauren P: The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histological classification. *Acta Pathol Microbiol Scand* 1965;64:31–49.
- 21 Sugano H, Nakamura K, Kato Y: Pathological studies of human gastric cancer. *Acta Pathol Jpn* 1982;32(suppl 2):329–347.
- 22 Jinawath N, Furukawa Y, Hasegawa S, Li M, Tsunoda T, Satoh S, Yamaguchi T, Imamura H, Inoue M, Shiozaki H, Nakamura Y: Comparison of gene-expression profiles between diffuse and intestinal-type gastric cancers using a genome-wide cDNA microarray. *Oncogene* 2004;23:6830–6844.
- 23 Watari J, Saitoh Y, Fujiya M, Shibata N, Tanabe H, Inaba Y, Okamoto K, Maemoto A, Ohta T, Yasuda A, Ayabe T, Ashida T, Yokota K, Obara T, Kohgo Y: Reduction of syndecan-1 expression in differentiated type early gastric cancer and background mucosa with gastric cellular phenotype. *J Gastroenterol* 2004;39:104–112.
- 24 Mizoshita T, Inada K, Tsukamoto T, Nozaki K, Joh T, Itoh M, Yamamura Y, Ushijima T, Nakamura S, Tatematsu M: Expression of the intestine-specific transcription factors, Cdx1 and Cdx2, correlates shift to an intestinal phenotype in gastric cancer cells. *J Cancer Res Clin Oncol* 2004;130:29–36.
- 25 Takahashi H, Endo T, Yamashita K, Arimura Y, Yamamoto H, Sasaki S, Itoh F, Hirata K, Imamura A, Kondo M, Sato T, Imai K: Mucin phenotype and microsatellite instability in early multiple gastric cancers. *Int J Cancer* 2002;100:419–424.
- 26 Line BR, Feustel PJ, Festin SM, Andersen TT, Dansereau RN, Lukasiewicz RL, Zhu S, Bennett JA: Scintigraphic detection of breast cancer xenografts with Tc-99m natural and recombinant human alpha-fetoprotein. *Cancer Biother Radiopharm* 1999;14:485–494.