Virchows Arch (1999) 435:112–115

ORIGINAL ARTICLE

Yun-Cai Cai · Zhong Jiang · Frank Vittimberga
Xiaowen Xu · Lou Savas · Bruce Woda
Mark Callery · Barbara Banner

Expression of transforming growth factor-α and epidermal growth factor receptor in gastrointestinal stromal tumours

Received: 29 December 1998 / Accepted: 30 March 1999

Abstract Activation of epidermal growth factor receptor (EGFR) is associated with cell growth and transformation. Both transforming growth factor-α (TGF-α) and epidermal growth factor bind to and activate EGFR. We studied the expression of TGF-α and two EGFRs (HER-1 and HER-2) in gastrointestinal stromal tumours (GISTs) of the stomach (*n*=9) and small intestine (*n*=6) using standard immunostaining techniques in paraffin-embedded sections. Most GISTs expressed TGF-α, and a few expressed HER-1. All HER-1-positive tumours expressed TGF-α. These results suggest that a TGF-α/EGFR autocrine loop is present in GIST and that TGF-α promotes proliferation of GIST tumour cells through its interaction with HER-1 in at least some GISTs. This is the first description of an autocrine loop in GIST. In contrast, HER-2 is not expressed in any GIST.

Key words GIST · TGF-α · HER-1 · HER-2 · Autocrine loop

Introduction

The intermediate filament components of gastrointestinal stromal tumours (GISTs) have been extensively studied, permitting their subclassification as smooth muscle, autonomic nerve cell, or CD34+ unclassified stromal cell subtypes [3, 6, 17]. Recently, Hirota showed expression of c-*kit* in GISTs and suggested an origin from the pacemaker cells [8]. However, the expression of an autocrine loop such as TGF-α and EGFR, which might influence cell proliferation, has not been well characterized. It has been shown that activation of EGFR by TGF-α is associated with cell growth and transformation in other systems [9, 13, 15, 22]. In vitro studies showed that disruption of this autocrine loop or suppression of EGFR expression resulted in growth arrest of tumour cells, stressing the importance of this autocrine loop in tumour growth and possibly in oncogenesis [5, 14, 18]. At least four members of the EGFR family have been characterized, including human HER-1 (EGFR-1, ErbB-1), HER-2 (neu, ErbB-2), HER-3 (ErbB-3), and HER-4 (ErbB-4) [2]. Since there is no previous report concerning this loop in GISTs, we collected 15 resected GISTs in our surgical pathology files to study the expression of these autocrine growth factors, including TGF-α, HER-1, and HER-2. The majority expressed TGF-α and a few cases were definitely positive for HER-1, suggesting that this TGF-α/EGFR loop exists and promotes tumour cell growth in at least some GISTs. However, HER-2 was not detected in any of these cases.

Y.-C. Cai · Z. Jiang · L. Savas · B. Woda · B. Banner (✉)
Department of Pathology,
University of Massachusetts Memorial Health Care,
Worcester, MA 01655, USA
Tel.: +1-508 856-3441
Fax: +1-508 856-2968

F. Vittimberga · M. Callery
Department of Surgery,
University of Massachusetts Memorial Health Care,
Worcester, MA 01655, USA

X. Xu
Department of Occupational Medicine,
University of Massachusetts Memorial Health Care,
Worcester, MA 01655, USA

Materials and methods

All resected GISTs were obtained from the surgical pathology files from January 1990 to August 1997 at UMMHC. Three small intestinal GISTs were excluded, one of which was 1 cm in size and had insufficient tissue left for study, while two were metastatic liver tumours with no primary tumour tissue available. The routine H&E-stained sections were reviewed to verify the diagnosis, to count mitoses, and to select a representative block for immunostaining. Based on previous studies [17], histological features noted were: presence of spindle and/or epithelioid cells, presence of necrosis, increased nuclear/cytoplasmic ratio, and pushing vs. infiltrating borders. Tumour site, size, clinical history, presence of metastases and outcome were obtained from the pathology reports and the patients' records. For immunostaining, sections from the paraffin blocks were cut at 4 μm, microwaved with an antigen retrieval solution and stained with an automated immunostainer using an avidin/biotin complex staining procedure. The primary anti-

Table 1 Antibodies used with their dilutions and sources

	Dilution	Source
TGF-α	1:500	Oncogene
HER-1	1:20	Novocastra
HER-2	1:40	Novocastra
Ki67	1:100	Immunotech
Vimentin	1:50	Dako
Smactin	1:100	Dako
CD34	1:100	Immunotech
S100	1:100	Chemicon
Desmin	1:50	Dako
GFAP	1:3000	Biogenex

bodies are shown in Table 1. Both positive and negative controls were included during each test. The positive control was normal breast ductal cells for TGF-α, squamous cell carcinoma of the skin for HER-1, and breast ductal carcinoma for HER-2. Tumour cells were called positive or negative for: vimentin, smooth muscle actin, CD34, S100, desmin and GFAP. Mitoses were counted in 10 high-power fields. Ki67+ nuclei were counted in 10 HPF, and expressed as the number positive per HPF. Positive staining for TGF-α, HER-1 and HER-2 was graded as 0 (negative), 1 (<25% of cells positive), 2 (25–50%), and 3 (>50%). Grades 2 and 3 were taken as positive staining. The immunohistochemical staining was evaluated by at least two pathologists independently.

Results

Characterization of GISTs

We studied 9 gastric and 6 small intestinal (SI) GISTs (Table 2). The ages of the patients ranged from 31 to 85 years, with a male predominance of gastric GISTs (6 of 9) and only men with SI GISTs. Tumours ranged in size from 0.5 to 15 cm in the stomach (mean=5) and from 3 to 23 cm in the SI (mean=9.5). The number of mitoses per 10 HPF ranged from 0 to 10 for stomach and from 0 to 9 for SI (Table 2). The proliferative rate, as detected by Ki67+ nuclei, was 0.1–19/HPF for gastric and 1.3–68.7/HPF for SI tumours. All tumours were spindle-cell type, with neither mucosal infiltration nor direct invasion of adjacent organs. Four cases were considered to be definitely malignant, because metastases were documented in either the liver or the peritoneum. Four additional cases were also defined as malignant based on the presence of two or more of four parameters (size >5 cm; tumour necrosis; increased nuclear-to-cytoplasmic ratio, and mitotic rates of >1 per 10HPF) [17]. All specimens stained positive for vimentin and negative for S100, desmin and GFAP. The majority showed focal immunoreactivity for smooth muscle actin (9 cases) and CD34 (14 cases).

Expression of TGF-α

Immunoreactivity for TGF-α was demonstrated in most GISTs (3+ in 80% and 2+ in 13% of cases) (Table 2). TGF-α was localized mainly in the cytoplasm (Fig. 1A). All 6 intestinal GISTs showed 3+ staining, and 6 of 9 gastric GISTs were 3+ positive and 2 were 2+ (Table 2). The expression levels of TGF-α did not correlate with tumour size, proliferative rate, or mitotic figures.

Expression of HER-1 and HER-2

A fraction of GISTs (33%) stained positive for HER-1, including one 3+ and two 2+ in stomach and two 3+ in the SI (Table 2). The staining was mainly cytoplasmic (Fig. 1B). Membranous staining was not detected. There was no expression in fibrous or smooth muscle tissues around tumours. Strong staining in GISTs was observed only in tumours with high expression of TGF-α, suggesting that HER-1 is co-expressed with TGF-α. There was

Table 2 Clinical, pathological and immunohistochemical features of 15 gastrointestinal stromal tumours (*G* stomach, *SI* small intestine, *Mets* metastases present around the time of resection in liver (cases 2, 10 and 13) or peritoneum (cases 2, 13 and 14), *Y* yes, *N* no, *ND* not done, *Mits* number of mitoses per 10 high-power fields, *Ki67* number of Ki67+ nuclei per high-power field, *Nec* coagulative necrosis, N/C↑ increased nuclear-cytoplasmic ratio, *Mal* malignancy, *Outcome* intervals (months) since the specimen submitted/ the most recent survey, *A(D)WOD* alive (dead) without disease, *A(D)WD* alive (dead) with disease)

Case	Age (years)/sex	Site	Mets	Size (cm)	Mits	Ki67	Nec	N/C↑	Mal	Outcome	TGF-α grade	HER-1 grade
1	31/M	G	N	2.9	10	16.4	N	N	N	66/AWOD	3	2
2	40/F	G	Y	0.5	1	11.5	N	N	Y	48/AWD	3	3
3	73/M	G	N	3.2	3	19	Y	N	Y	2/AWOD	1	1
4	38/M	G	N	6.5	2	0.1	N	N	Y	15/AWOD	2	0
5	44/F	G	N	6	1	6.4	N	Y	Y	15/AWOD	2	1
6	74/M	G	N	15	1	2.9	N	N	N	2/DWOD	3	2
7	84/M	G	N	4	0	4	N	N	N	5/AWOD	3	1
8	62/F	G	N	1.5	1	2	N	Y	N	60/AWOD	3	1
9	85/M	G	N	ND	0	0.5	N	N	N	26/AWOD	3	1
10	33/M	SI	Y	11.4	5	42.7	Y	N	Y	2/AWD	3	3
11	61/M	SI	N	5	2	6.5	Y	N	Y	24/AWOD	3	0
12	47/M	SI	N	3	2	1.3	N	N	N	1/AWOD	3	0
13	45/M	SI	Y	11	2	44.2	Y	N	Y	1/AWD	3	0
14	65/M	SI	Y	23	9	68.7	Y	Y	Y	6/AWD	3	3
15	57/M	SI	N	4	0	28	N	N	N	1/AWOD	3	1

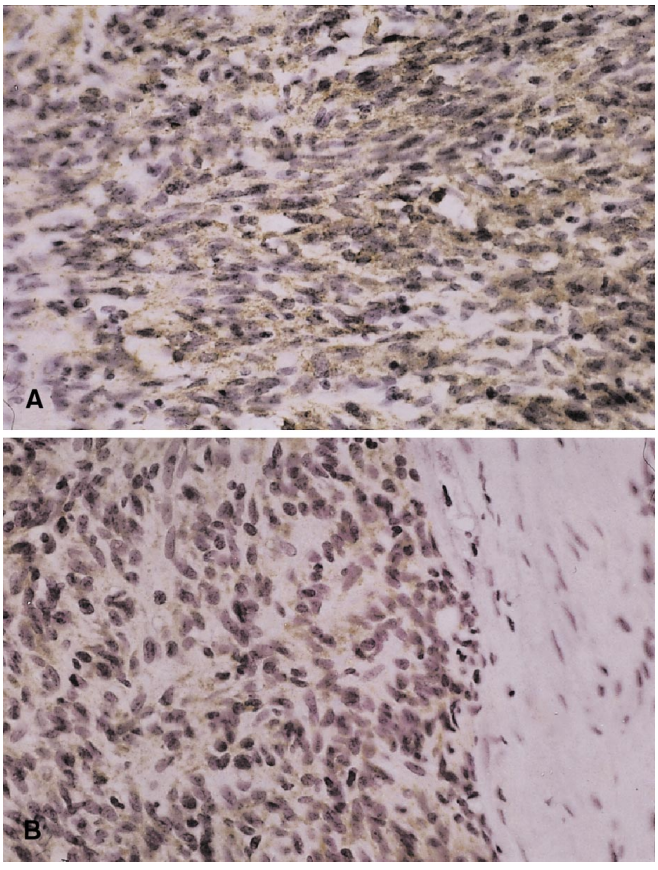

Fig. 1 **A** Section immunostained for TGF-α, showing cytoplasmic expression in tumour cells. Original magnification ×400. **B** Section immunostained for HER-1, showing cytoplasmic expression in tumour cells. Original magnification ×400

no correlation between HER-1 expression and the tumour size or proliferative rate. In contrast to HER-1, HER-2 was not detected on the membrane or in the cytoplasm in any of these cases.

Discussion

A TGF-α/EGFR autocrine loop plays an important role in tumour growth. TGF-α is a mitogenic polypeptide composed of 50 amino acids, which shares 42% homology with the prototype, EGF [11, 12, 19]. The precursor (pro-TGF-α) is a 160-amino-acid protein with a single transmembrane domain. It is cleaved to release the mature form, which binds and stimulates EGFR. EGFR has similarity to the transforming protein of the avian erythroblastosis virus (v-erb-B) at the amino acid level [4]. All four members of EGFR are synthesized in the cytoplasm and transported to the cell membrane, where they serve as receptors for at least seven ligands, EGF, TGF-α, amphiregulin, heparin-binding EGF-like growth factor, betacellulin, epiregulin, and neuregulins [2, 10]. Of the four known EGFRs, HER-1 binds to the first six ligands while HER-2 only binds to TGF-α after forming a heterodimer with HER-1 [1, 2]. The interaction between ligands and EGFR results in auto-tyrosine phosphorylation of EGFR, leading to activation of the Janus kinase (Jak2) and mitogen-activated protein (MAP) kinases [20]. These in turn regulate gene expression and promote cell growth. TGF-α and EGFR are expressed by the proliferating and differentiating cells, including gastrointestinal epithelial cells [7, 21]. The autocrine loop formed by TGF-α and EGFR has been demonstrated in malignant tumours, including carcinomas of lung, colon and breast [9, 13, 15]. The requirement of TGF-α for tumour cell growth was demonstrated by the blockage of the interaction between TGF-α and its receptor, EGFR [5, 14]. Down-regulation of EGFR was concomitant with tumour growth arrest in another in vitro experiment [18]. Similarly, over-expression of TGF-α in transgenic mice resulted in the development of breast cancer [16]. These studies emphasize the important role of EGFR in tumour proliferation. This report is the first description of the existence of this autocrine loop in GIST.

Immunoreactivity for TGF-α and HER-1 was observed mainly in the cytoplasm of the tumour cells. In contrast, the membranous staining for controls was stronger than the cytoplasmic staining. Thus, the same antibody stained differently in different tumours. The low membranous expression of both TGF-α and HER-1 in GIST could be due to a slow transport from the cytoplasm to the cell membrane or a rapid turnover rate from the cell membrane. Alternatively, they may be configured in some way in the membrane or altered by fixation in such a way that it was difficult to detect their expression by immunostaining techniques. However, low expression of HER-1 may still be sufficient to form an autocrine loop. This has implications for development of possible future therapies, in that targeting this loop might benefit the treatment of GIST. In contrast to HER-1, HER-2 was not detectable in any of these cases.

In summary, in a series of 9 gastric and 6 SI GISTs, TGF-α was demonstrated in all of the intestinal and 89% of the gastric (2+ and 3+) GISTs. HER-1 was also manifested in 33% of gastric (one 3+ and two 2+) and 33% of SI GISTs (3+ only). HER-2 was not detected. This is significant because it implies the presence of an autocrine loop of TGF-α/HER-1 in a fraction of GISTs. The lack of correlation between their expression and mitoses or proliferation rates indicates that factors other than TGF-α/EGFR also influence tumour cell proliferation.

References

1. Beerli RR, Hynes NE (1996) Epidermal growth factor-related peptides activate distinct subsets of ErbB receptors and differ in their biological activities. J Biol Chem 271:6071–6076
2. Brown KD (1995) The epidermal growth factor/transforming growth factor-α family and their receptors. Eur J Gastroenterol Hepatol 7:914–922
3. Dhimes P, Lopez-Carreira M, Ortega-Serrano MP, Garcia-Munoz H, Martinez-Gonzalez MA, Ballestin C (1995) Gastrointestinal autonomic nerve tumours and their separation from other gastrointestinal stromal tumours: an ultrastructural and

immunohistochemical study of seven cases. Virchows Arch 426:27–35
4. Downward J, Yarden Y, Mayes E, Scrace G, Totty N, Stockwell P, Ullrich A, Schlessinger J, Waterfield MD (1984) Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. Nature 307:521–527
5. Ennis BW, Valverius EM, Bates SE, Lippman ME, Bellot F, Kris R, Schlessinger J, Masui H, Goldenberg A, Mendelsohn J, Dickson RB (1989) Anti-epidermal growth factor receptor antibodies inhibit the autocrine-stimulated growth of MDA-468 human breast cancer cells. Mol Endocrinol 3:1830–1838
6. Franquemont DW (1995) Differentiation and risk assessment of gastrointestinal stromal tumours. Am J Clin Pathol 103: 41–47
7. Fukuyama R, Shimizu N (1991) Expression of epidermal growth factor (EGF) and the EGF receptor in human tissues. J Exp Zool 258:336–343
8. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Muhammad TG, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y (1998) Gain-of-function mutations of c-kit in human gastrointestinal stromal tumours. Science 279:577–580
9. Karnes WE Jr, Walsh JH, Wu SV, Kim RS, Martin MG, Wong HC, Mendelsohn J, Park J-G, Cuttitta F (1992) Autonomous proliferation of colon cancer cells that coexpress transforming growth factor α and its receptor. Gastroenterology 102:474–485
10. Komurasaki T, Toyoda H, Uchida D, Morimoto S (1997) Epiregulin binds to epidermal growth factor receptor and ErbB-4 and induces tyrosine phosphorylation of epidermal growth factor receptor, ErbB-2, ErbB-3 and Erb-B-4. Oncogene 15: 1841–1848
11. Marquardt H, Hunkapiller MW, Hood LE, Todaro GJ (1984) Rat transforming growth factor type 1: structure and relation to epidermal growth factor. Science 223:1079–1082
12. Massague J (1983) Epidermal growth factor-like transforming growth factor. II. Interaction with epidermal growth factor receptors in human placenta membranes and A431 cells. J Biol Chem 258:13614–13620
13. Pilichowska M, Kimura N, Fujiwara H, Nagura H (1997) Immunohistochemical study of TGF-alpha, TGF-beta, EGFR, and IGF-1 expression in human breast carcinoma. Mod Pathol 10:969–975
14. Rodeck U, Noel W, Murthy U, Herlyn M (1990) Monoclonal antibody 425 inhibits growth stimulation of carcinoma cells by exogenous EGF and tumour-derived EGF/TGF-alpha. J Cell Biochem 4:69–79
15. Rusch A, Baselga J, Cordon-Cardo C, Orazem J, Zaman M, Hoda S, McIntosh J, Kurie J, Dmitrovsky E (1993) Differential expression of the epidermal growth factor receptor and its ligands in primary non-small-cell lung cancers and adjacent benign lung. Cancer Res 53:2379–2385
16. Sandgren EP, Luetteke NC, Palmiter RD, Brinster RL, Lee DC (1990) Overexpression of TGF-alpha in transgenic mice: induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. Cell 61:1121–1135
17. Suster A (1996) Gastrointestinal stromal tumours. Semin Diagn Pathol 13:297–313
18. Szepeshazi K, Schally AV, Halmos G, Lamharzi N, Groot K, Horvath JE (1997) A single in vivo administration of bombesin antagonist RC-3095 reduces the levels and mRNA expression of epidermal growth factor receptors in MXT mouse mammary cancers. Proc Natl Acad Sci USA 94:10913–10918
19. Todaro GJ, Fryling C, DeLarco JE (1980) Transforming growth factors produced by certain human tumour cells: polypeptides that interact with epidermal growth factor receptors. Proc Natl Acad Sci USA 77:5258–5262
20. Yamauchi T, Ueki K, Tobe K, Tamemoto H, Sekine N, Wada M, Honjo M, Takahashi M, Takahashi T, Hirai H, Tushima T, Akanuma Y, Fujita T, Komuro I, Yazaki Y, Kadowaki T (1997) Tyrosine phosphorylation of the EGF receptor by the kinase Jak2 is induced by growth hormone. Nature 390:91–96
21. Yasui W, Ji Z-Q, Kuniyasu H, Ayhan A, Yokozaki H, Ito H, Tahara E (1992) Expression of transforming growth factor alpha in human tissues: immunohistochemical study and Northern blot analysis. Virchows Arch [A] 421:513–519
22. Yoshida K, Hosoya Y, Sumi S, Honda M, Moriguchi H, Yano M, Ueda Y (1997) Studies of the expression of epidermal growth factor receptor in human renal cell carcinoma: a comparison of immunohistochemical method versus ligand binding assay. Oncology 54:220–225