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Case report

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Transforming growth factor-alpha (TGF α)-producing gastric carcinoma with acanthosis nigricans: An endocrine effect of TGF α in the pathogenesis of cutaneous paraneoplastic syndrome and epithelial hyperplasia of the esophagus

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Abstract: A case of well-differentiated adenocarcinoma (Borrmann type 3) of the stomach in a 76-year-old man associated with the typical skin manifestations of acanthosis nigricans and with multiple protruding lesions showing epithelial hyperplasia of the esophagus is reported. The advanced tumor was located in the cardiac region of the stomach, and measured approximately 8 cm in diameter, with partial invasion to the esophagus. The associated cutaneous lesions were characterized by hyperpigmentation and by protruding verrucous papules on the torso, head, face, neck, upper extremities, perineum, and inguinal region. Histologically, the protruding skin lesions showed keratinocytes proliferation throughout the epidermis, resulting in diffuse hyperkeratosis, papillomatosis, and acanthosis of the skin. Immunohistological analysis showed coexpression of transforming growth factor alpha (TGF-α) and epidermal growth factor (EGF) receptors in the tumor from the stomach. It is reasonable to conclude from this evidence that gastric carcinoma cells secrete TGF α in an autocrine for auto-stimulation. EGF receptor expression was also noted on the papillomatous hyperplasia of the cutaneous lesion. Serum level of TGF α, determined by an enzyme-linked immunosorbent assay, was high (144 pg/ml; normal, 22.0 ± 16 pg/ml (Mean ± SD)). Serum TGF α abruptly decreased to 49 pg/ml on day 7 after the total gastrectomy, and then gradually increased to 77 pg/ml within 28 days. Amelioration of the cutaneous lesions and the protruding lesions in the esophagus was observed after surgical resection of the gastric carcinoma. This suggests that the TGF α stimulates the proliferation of keratinocytes involved with EGF receptor. Large amounts of circulating TGF α in the blood over a long period released by the primary tumor seem to act as an endocrine-like mechanism causing epidermal and esophageal epithelial cells to proliferate. There is a possible link in the pathogenesis of the acanthosis nigricans as a cutaneous paraneoplastic syndrome, and epithelial hyperplasia of the esophagus.

Key words: acanthosis nigricans, transforming growth factor-alpha (TGF α), epidermal growth factor (EGF) receptor, gastric carcinoma

Introduction

Acanthosis nigricans is a rare cutaneous disorder characterized by hyperkeratosis and pigmentation; the affected skin is covered by papillomatous elevations that appear on the neck, arms, and in the axillae and other body folds. This type of cutaneous change can occur in association with endocrine disturbances, underlying malignant disease, the administration of some drugs and hormones, or as an inherited disorder. Cases linked with malignancy have been called malignant acanthosis nigricans; other cases are termed benign.[1] Here we are concerned only with the form associated with malignancy. Acanthosis nigricans is most often associated with adenocarcinoma, generally of the stomach, but also appearing elsewhere in the gastrointestinal tract. Ovarian, prostatic, breast, and lung carcinomas, are also reported to be associated, and there are rare instances of lymphoma and squamous cell carcinoma being associated with acanthosis nigricans.[1]

Although the cause of skin changes in malignant acanthosis nigricans is still obscure, it appears likely that

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it will ultimately prove to be due to a circulating humoral epidermotrophic factor released by the tumor, since the cutaneous lesions have been shown to be proliferative disorders of keratinocytes and to disappear after surgical reduction of the tumor mass,[1] as was observed in the patient reported here. In this context, Ellis et al.[2] reported a case of malignant acanthosis nigricans associated with elevated transforming growth factor alpha (TGF α) levels in urine. Of etiological relevance to the cutaneous paraneoplastic syndrome of malignant acanthosis nigricans, Wilgenbus et al.[3] showed amplification of epidermal growth factor (EGF) receptor expression in gastric carcinoma, and immunohistochemical expression of TGF α and EGF receptors in the tumor. However, serum levels of circulating TGF α in patients with similar cutaneous lesions have not been determined, possibly for technical reasons.[2,3]

To further investigate the pathogenesis of this rare skin disease, we present a case of TGF α-producing advanced gastric carcinoma associated with multiple epithelial hyperplastic lesions of the esophagus, together with cutaneous paraneoplastic syndrome, which was categorized as malignant acanthosis nigricans. TGF α and its receptor have been reported to play an important role in tumor progression via autocrine or paracrine secretion for auto-stimulation.[4,5] In the present study, the possible endocrinous effect of high level of TGFα which were released by the primary tumor of the stomach was examined in the pathogenesis of acanthosis nigricans and epithelial hyperplasia of the esophagus. Our results confirmed and extended the findings reported by Ellis et al.[2] and Wilgenbus et al.[3]

Case report

A 76-year-old man was admitted to our University hospital in June 1995 presenting with appetite loss and hyperpigmented skin on the face and neck, and virtually the entire body. The cutaneous lesion had not been treated, although the patient noticed the hyperpigmentation of the face in April 1993 and the small protruding lesions, approximately 5 mm in diameter, on the back of the hands and arms in April 1994. As the protruding lesions and hyperpigmentation of the skin were exacerbated and appeared in the axillae, neck, and inguinal region around February 1995, the patient visited our hospital. On physical examination, the patient was a relatively well-nourished man (height 165 cm, weight 58.6 kg) who was afebrile, with a heart rate of 62/min (regular), and blood pressure of 136/76 mmHg. There were no physical abnormalities of the abdomen, except

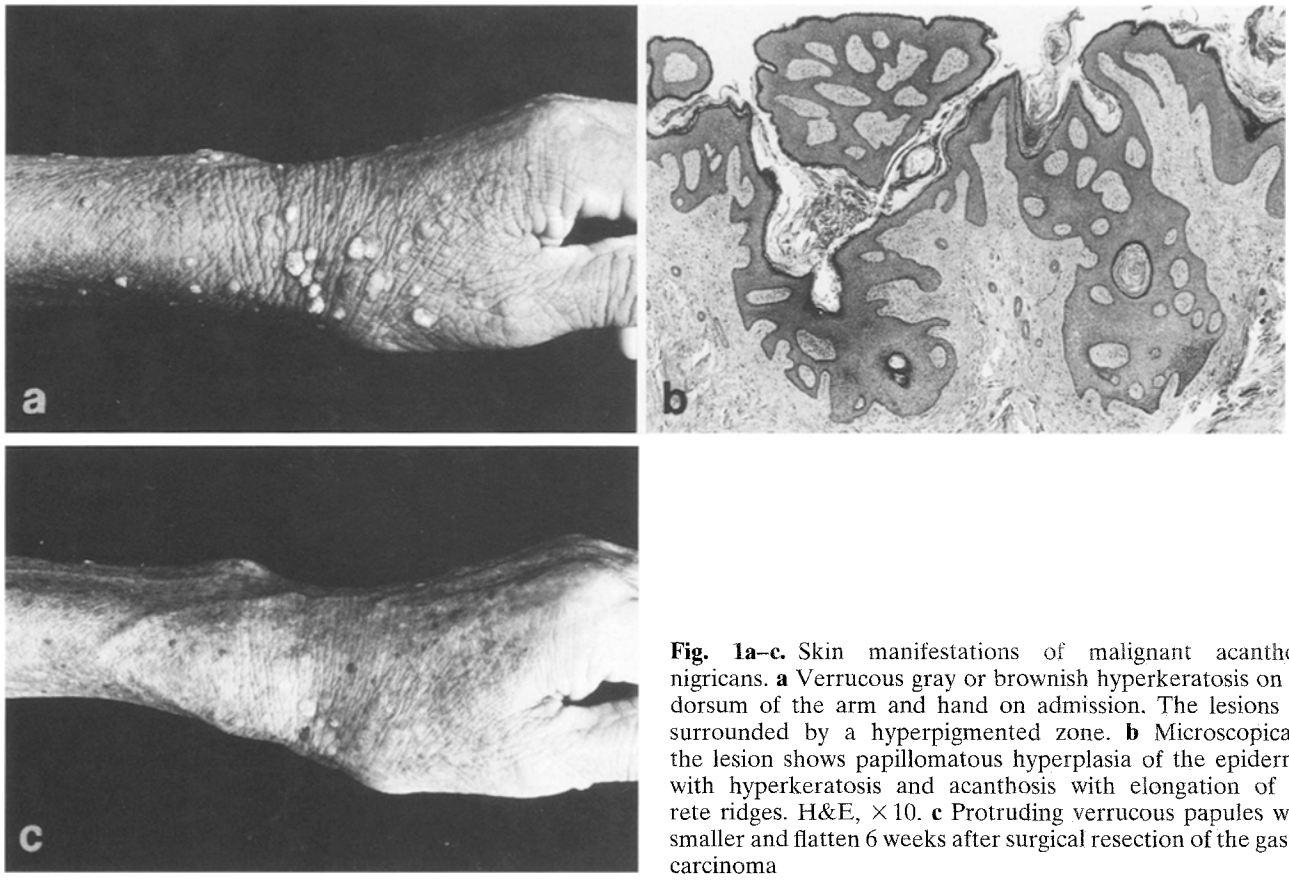

Fig. 1a–c. Skin manifestations of malignant acanthosis nigricans. **a** Verrucous gray or brownish hyperkeratosis on the dorsum of the arm and hand on admission. The lesions are surrounded by a hyperpigmented zone. **b** Microscopically, the lesion shows papillomatous hyperplasia of the epidermis, with hyperkeratosis and acanthosis with elongation of the rete ridges. H&E, ×10. **c** Protruding verrucous papules were smaller and flatten 6 weeks after surgical resection of the gastric carcinoma

for the cutaneous manifestations. Hyperpigmentation and dryness and roughness of the skin were seen on the torso, head, face, neck, upper extremities, perineum, and inguinal region. The affected areas were gray-brown or black, palpably thickened and covered by small papillomatous elevations that gave the skin a velvety texture. The lateral and nape regions of the neck, the axillae, and the dorsum of arms and hands (Fig. 1a) were involved with verrucous papules. These findings, however, were not observed on the lips, buccal mucosa, and tongue. Histologically, the epithelial cells of the epidermis (keratinocytes) taken from the pigmented and verrucous lesions on the back of the arms, showed marked keratinocytes proliferation, with an undulating lower border and with downward growth. There was considerable hyperkeratosis, papillomatosis, and acanthosis, but hypermelanization was minimal (Fig. 1b).

Relevant laboratory data on admission were: RBC 411 × 10^4/μl, Hb 12.5 g/dl, Ht 37.5%, WBC 8500/μl (64% neutrophils, 25% lymphocytes, 3% monocytes, 4% eosinophils, 1% basophils), and platelet count 28.4 × 10^4/μl. Serum protein level was 6.5 g/dl. Serum GOT was 19 IU/l, GPT 13 IU/l, LDH 363 IU/l, ALP 281 IU/l, γ-GTP 32 IU/l, CHE 244 IU/l, total bilirubin 0.5 mg/dl, BUN 19.3 mg/l, creatinine 0.8 mg/dl, and CRP 0.3 mg/dl. Serological tumor markers (AFP, CEA, CA19-9, SCC, and NSE) were negative. Radiological and endoscopic examinations of the upper gastrointestinal (GI) tract showed Borrmann type 3 tumor (Fig 2a), measuring approximately 8 cm in diameter, located in cardiac region of the stomach, with some invasive growth in the esophagus. Biopsy taken from the lesion showed well differentiated tubular adenocarcinoma. In addition, multiple protruding lesions were found throughout the esophagus (Fig. 2b). The histological findings of the lesion were squamous epithelial hyperplasia (Fig. 2c). Abdominal computed tomography (CT) and sonograms showed tumor metastasis to regional lymph

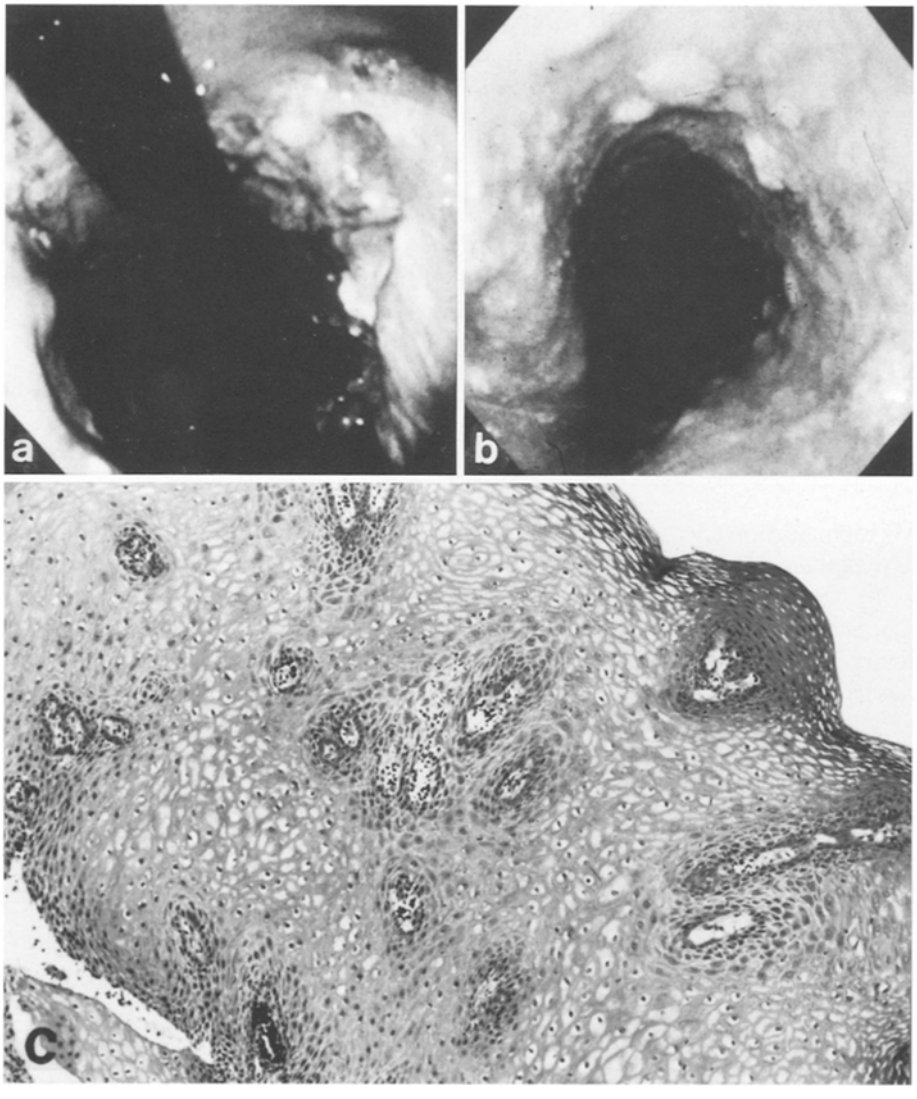

Fig. 2a–c. Examination of the upper gastrointestinal (GI) tract. **a** Endoscopic findings in J-turn view show Borrmann type-3 tumor in the cardiac region of the stomach. **b** Endoscopic findings show multiple protruding lesions of the esophagus. **c** Biopsy specimen from the protruding lesion in the esophagus shows epithelial hyperplasia. H&E, ×25

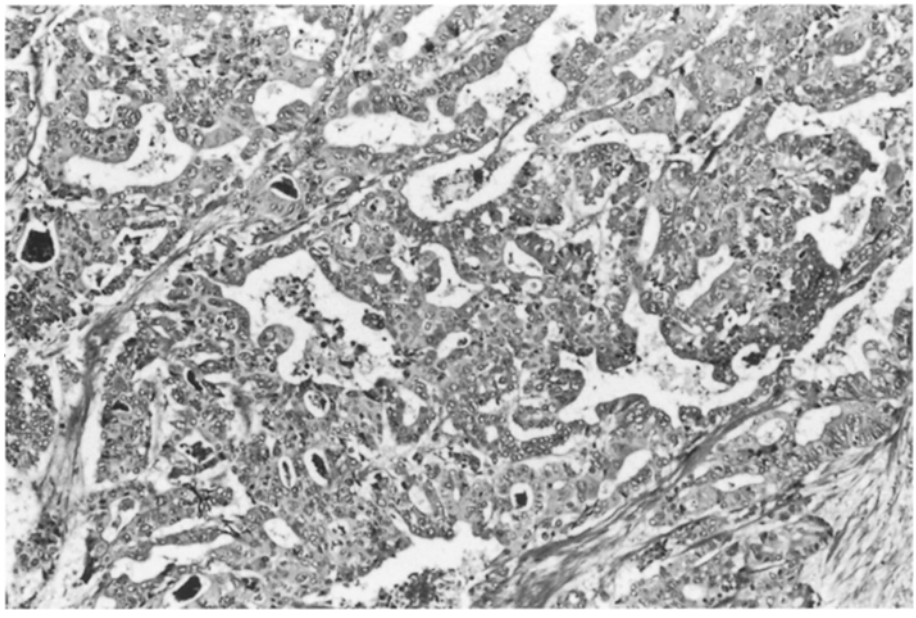

Fig. 3. Microscopic findings of the resected stomach, showing well differentiated adenocarcinoma. H&E, ×25

nodes, but not metastasis to the liver. Based on these findings, tubular adenocarcinoma of the stomach associated with typical acanthosis nigricans was diagnosed. The patient underwent total gastrectomy for proximal gastric cancer, associated with pancreaticosplenectomy, on July 1995. Resected materials in the surgical specimen of the stomach showed advanced Borrmann type 3 carcinoma, measuring 8 × 9 cm, with some invasion of the esophagus, located in the cardiac wall of the stomach. Histologically, the tumor was well differentiated tubular adenocarcinoma (Fig. 3) infiltrating to the subserosa. The lesion in the stomach showed cancer cells involving lymphatic and vascular venous vessels. Metastasis involving the perigastric lymph nodes was also found histologically. Gross staging, according to the guidelines of the Japanese Research Society for Gastric Cancer,[6] and TNM staging,[7] showed t2(ss)n2P0H0 (stage IIIa) and T2N2M0 (stage IIIA), respectively.

To perform further immunohistochemical analysis, fresh surgical specimens from the primary gastric carcinoma and the papillomatous verrucous skin lesions on the dorsum of the arms were fixed with periodate-lysine-paraformaldehyde (PLP). These PLP-fixed samples were embedded in OCT compound and stained with monoclonal anti TGF α (Wakunaga Pharmaceutical, Tokyo, Japan) and monoclonal anti EGF receptor (Kyokuto Pharmaceuticals, Tokyo, Japan) by immunoperoxidase staining by the avidin-biotin-complex method (Vectastain ABC kit, Vector, Burlingame, CA). Phosphate-buffered saline (PBS) instead of primary antibody was used on duplicate slides from serial sections as a control in the experiments. Figure 4a,b shows the strong expression of TGF α and EGF receptor in the primary gastric carcinoma. The densely aggregated skin lesions showing papillomatous hyperplasia throughout the epidermis were positively stained with anti-EGF receptor (Fig. 4c). However, TGF α was not detected in the cutaneous lesions in the same analysis.

Serum levels of TGF α in the patient before and after total gastrectomy were quantitatively determined by an enzyme-linked immunosorbent assay (ELISA), as originally described by Tomiya and Fujiwara.[8] Blood was collected before surgery and on days 7, 14, 21, and 28 after surgery. As shown in Fig. 5, serum TGF α level before surgery was 144 pg/ml; the level abruptly decreased within 7 days after surgery, to 49 pg/ml. However, the level gradually increased within 28 days of surgery. The cutaneous lesions, as a paraneoplastic syndrome, in particular the verrucous papules on the arms, regressed and were smaller and flatten around 4 weeks after surgery. Figure 1c shows amelioration of the skin lesions on the arms 6 weeks after the surgery. Similarly, amelioration of the protruding lesions of the esophagus was observed on roentgenological examination of the upper GI tract after the surgery. Hyperpigmentation of the skin, however, remained unchanged at this time after the surgery.

Discussion

We clearly showed that TGF α, which was produced and released by gastric carcinoma, not only caused the autocrine growth of the primary tumor, but also promoted the proliferation of the epithelial cells of epidermis and the squamous cells via an endocrine-like

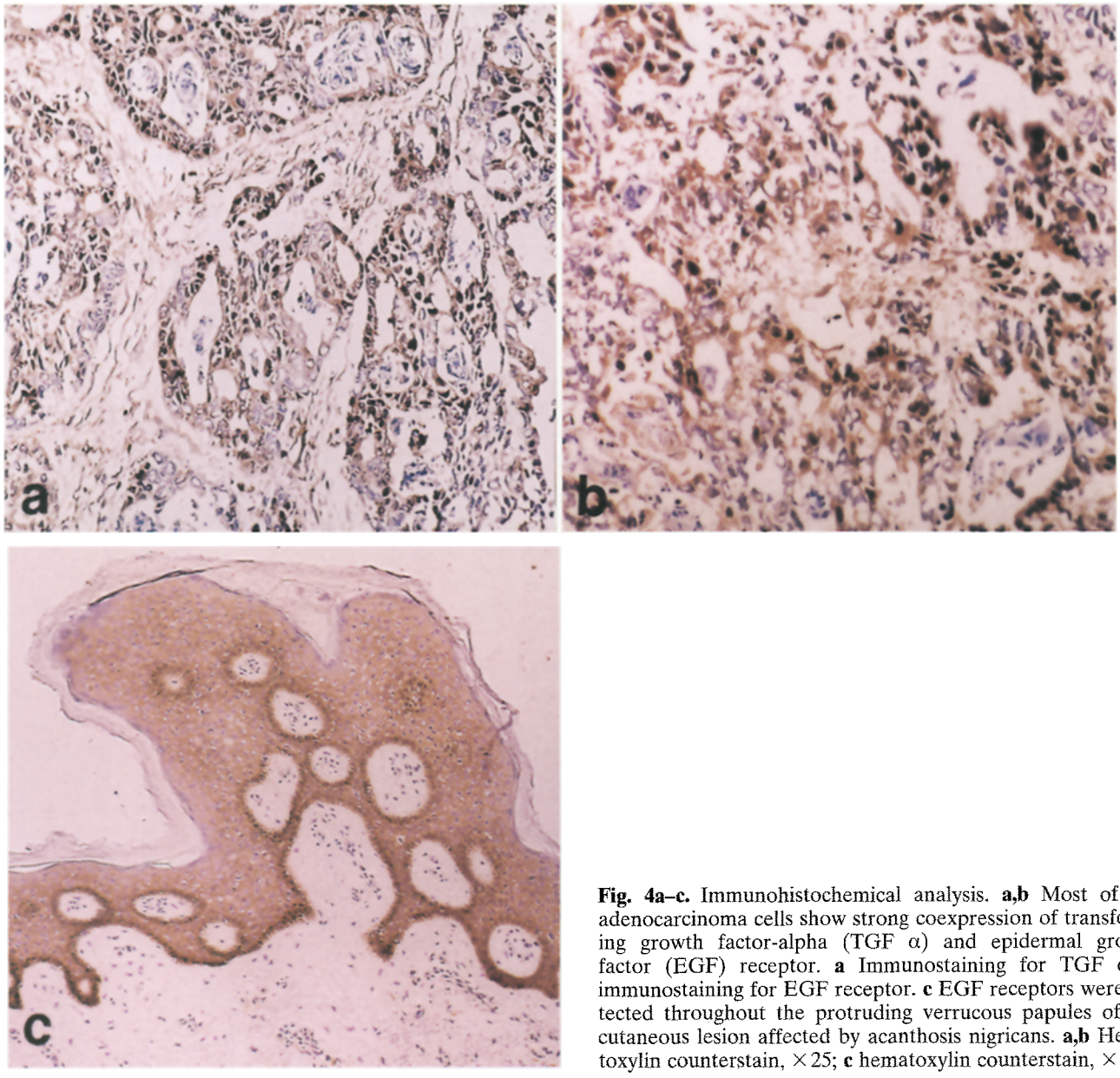

Fig. 4a–c. Immunohistochemical analysis. **a,b** Most of the adenocarcinoma cells show strong coexpression of transforming growth factor-alpha (TGF α) and epidermal growth factor (EGF) receptor. **a** Immunostaining for TGF α; **b** immunostaining for EGF receptor. **c** EGF receptors were detected throughout the protruding verrucous papules of the cutaneous lesion affected by acanthosis nigricans. **a,b** Hematoxylin counterstain, ×25; **c** hematoxylin counterstain, ×10

mechanism. That is, TGF α appeared to play a role in the pathogenesis of cutaneous paraneoplastic syndrome and the multiple protruding lesions of the esophagus.

TGF α is structurally related to EGF and shares the same cell surface receptor, although probably interacting with different sites.[9] These molecules have been shown to be expressed in human gastric carcinoma.[4,5,10,11] Positive immunoreactivity of the EGF receptor was detected in 44 (33.8%) of 130 advanced gastric carcinomas in the report of Yasui et al.[10] EGF/EGF receptor coexpression was reported in 17 (13.1%) of these 130 advanced carcinomas. Yasui et al.[10] stated that synchronous expression of EGF/EGF receptor was correlated with the depth of tumor invasion, and, thus, these patients had a far poorer prognosis than those without EGF and its receptor. TGF α has been reported to be immunohistochemically detected in 52%–60% of advanced gastric carcinomas examined.[5,11] Synchronous expression of TGF α and EGF receptor was observed in 50 (30.5%) of 167 gastric cancers, and this was synchronous expression more frequent in larger tumors (measuring 6 cm or more) than in smaller tumors.[5] These findings reflect an autocrine mechanism involving TGF α/EGF and EGF receptor within gastric carcinoma. Namely, TGF α or EGF stimulates the autophosphorylation of EGF receptor.[12] In the present study, advanced Borrmann type 3 adenocarcinoma, measuring 8 × 9 cm, showed very intense immunoreactivity with both anti TGF α and anti-EGF receptor antibodies. Thus, the coexpression of TGF α/EGF

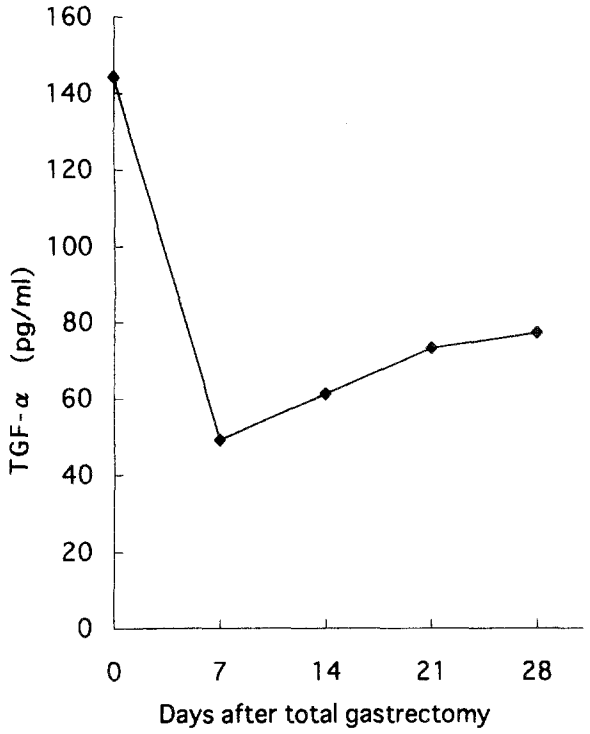

Fig. 5. Changes in serum TGF α level after surgical resection of the gastric carcinoma

receptor in the tumor suggests an aberrant autocrine loop for auto-stimulation, which may play an important role in the further progression of gastric carcinoma toward invasive tumor growth and metastatic potential.

TGF α and EGF receptor have also been shown to be expressed in normal human epithelial cells of the epidermis.[13–15] EGF receptor expression is primarily restricted to actively proliferating keratinocytes of the basal layer in normal skin.[13] These molecules in the keratinocytes play a role in epidermal homeostasis, for example in normal keratinocyte growth/differentiation and wound healing, through autocrine regulation. Interestingly, however, in our patient, EGF receptors were detected in the verrucous papules (as acanthosis nigricans) that were clinically documented to be actively growing (Fig. 1a,b), and these receptors were found throughout the epithelial cells of the epidermis in the samples examined (Fig. 4c), although EGF receptors in the gastric lesion were not always detected in all carcinoma cells observed. Similar changes in EGF receptor distribution have been reported in the resolving paraneoplastic skin lesions of a patient after excision of melanoma, and, coincident with this change in receptor pattern, urinary TGF α was also decreased.[2] The epidermotrophic factor responsible for our patient's skin lesions appeared to be TGF α released by the stomach tumor, but not TGF α produced by local keratinocytes, as indicated by findings of EGF receptor stimulation of the keratinocytes throughout the epidermis of the cutaneous lesions, and by the findings that increased levels of serum TGF α in the patient rapidly declined after the surgery, followed by clinical improvement of the cutaneous lesions.

In general, TGF α has been reported to work as an autocrine or paracrine growth factor on local sites of tumors involving EGF receptors.[4,5] For TGF-α to act in an endocrine fashion on its receptors in target cells a long distance from the stomach, such as those in the skin or mucosa of the esophagus, large quantities of TGF α would have to be produced by tumor cells themselves to counteract the effect of dilution of the growth factors in the circulation. Indeed, the level of circulating TGF α in our patient was high (144 pg/ml). Tomiya and Fujiwara[8] have shown that the normal range of serum TGF α in healthy adults was 22.0 ± 16 pg/ml (mean ± SD), and the serum levels of TGF α in 13 patients with hepatocellular carcinoma or metastatic liver tumor were less than 100 pg/ml. After partial hepatectomy, the serum TGF α levels increased transiently within 28 days in all patients, and this was regarded as indicating regeneration of the remaining hepatic cells. However, their patients were not reported to have any documented skin disease, despite having higher levels of serum TGF α than that in our patient. On the contrary, the high level of serum TGF α produced by the gastric carcinoma in our present patient was considered to have persisted for a long time, because the skin manifestation had been exhibited since April 1993. Taken together with the findings of Tomiya and Fujiwara,[8] therefore, it would be reasonable to assume that high levels of serum TGF α over a long time were a major cause of acanthosis nigricans as a cutaneous paraneoplastic syndrome. The same concept could be applied to the pathogenesis of the epithelial hyperplasia of the esophagus, since EGF receptor has been found in squamous epithelial cells of the esophagus.[16] Therefore, we presumed that the gastric carcinoma had been clinically present before the appearance of the protruding skin lesions.

Serum levels of TGF α rapidly decreased on day 7 after the suergery, followed by a gradual increase in the level within the following 28 days. The postoperative re-elevation of serum TGF α level may have been due to hepatic metastasis of the tumor cells via portal vein, or to the regrowth of remnant cancer cells in the abdominal lymph nodes, since serum TGF α levels are essentially dependent on the presence of TGF α-producing gastric carcinoma cells. Indeed, 9 months after the surgery, two metastatic lesions of the liver, each measuring 2 cm in diameter, were found by echography.

In conclusion, in this patient, the large quantity and the long-term duration of TGF α production and release by the tumor cells of the stomach seemed to be linked to the skin manifestations, as a paraneoplastic

syndrome, and to the epithelial hyperplasia of the esophagus.

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