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CASE REPORT

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Expression of insulin-like growth factor II by a gastric carcinoma associated with hypoglycaemia

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Abstract A gastric cancer with liver metastases was associated with low morning levels of plasma glucose (24 mg/dl), insulin (<2.5 μU/ml) and growth hormone (0.23 ng/ml). Primary and metastatic tumour tissue stained positively with anti-insulin-like growth factor II(IGF-II) monoclonal antibody. Western immunoblot analysis revealed a high molecular weight IGF-II in the serum: 15 kDa (normal: 7.5 kDa). Postmortem reverse transcription polymerase chain reaction on mRNA from both sites revealed 471 base pairs size cDNA encoding prepro-IGF-II. These results suggest that the gastric carcinoma encoded, expressed, and secreted IGF-II, probably causing the extrapancreatic tumour hypoglycaemia.

Key words Insulin-like growth factor II
Hypoglycaemia · Gastric cancer
Non-islet cell tumour hypoglycaemia · Insulin receptor

Introduction

The pathogenesis of hypoglycaemia in patients with non-islet cell tumour hypoglycaemia (NICTH) has been described extensively. Megyesi et al. (1974) reported elevated levels of non-suppressible insulin-like activity in NICTH patients; Daughaday et al. (1981) reported elevated insulin-like growth factor II (IGF-II) in 10 of 14 serum samples using the rat placental membrane and radioreceptor assay. In contrast, Zapf et al. (1981) found no elevation in serum IGF-II in similar patients, using radioimmunoassay (RIA), and Widmer et al. (1982) found no significant increase in serum IGF-II using rat liver plasma membrane. A later study by Daughaday et al. (1988) again found that high molecular weight IGF-II was present both in the serum and the tumour of a patient with leiomyosarcoma, suggesting the existence of incompletely- or unprocessed pro-IGF-II. We have used anti-IGF-II monoclonal antibody RIA to measure IGF-II serum levels, western blot analysis to measure the IGF-II molecular size, and reverse transcriptase polymerase chain reaction (RT-PCR) analysis of mRNA transcripts to determine the processing of IGF-II and the expression of IGF-II mRNA in gastric carcinoma with liver metastases and associated hypoglycaemia.

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Case report

An 82-year-old man was admitted to Teikyo University Ichihara Hospital in August, 1992. One week prior to admission, he had a sudden episode of diaphoresis and stupor which cleared after eating breakfast; this had recurred on the morning of admission. His plasma glucose level was 45 mg/dl; he appeared emaciated weighing 48 kg and measuring 165 cm in height. Physical examination revealed palpable hepatomegaly at the right costal margin and clubbed fingers. Plasma immunoreactive insulin was below 3.0 μU/ml; glucagon, 150 pg/ml; growth hormone (GH), 0.23 ng/ml; cortisol, 21 μg/dl; corticotropin, 30 pg/ml; thyroid stimulating hormone, 2.2 μU/ml; free thyroxine, 2.1 ng/dl; free tri-iodothyronine, 1.7 pg/ml. Serum cortisol and glucagon were elevated. Cathecholamines were within normal limits. Serum IGF-I and IGF-II were 27.1 and 1337 ng/ml, respectively: this IGF-I:IGF-II ratio (49) was extremely high. Abdominal computed tomography (CT) showed multiple liver metastases and a normal pancreatic shadow. Gastrofibrescopy revealed an irregular ulcerated lesion, macroscopically Borrmann's type III, in the antrum; biopsy revealed adenotubular carcinoma.

On the morning of hospital day 30, daily therapy of 4000 kCal intravenous hyperalimentation, with a somatostatin analogue (octreotide), was started. This resulted in slight symptomatic improvement, but no correction of the hypoglycaemia, and a slight increase in IGF-II. On day 100, he developed renal failure and died 2 days later.

Materials and methods

Specific RIA was used to determine the levels of IGF-II (Asakawa et al. 1990), IGF-I, insulin, and GH. IGF-II serum levels in this study ranged from 374 to 804 ng/ml.

For RT-PCR total cellular RNA was isolated from the gastric carcinoma cells and liver metastases, using guanidium thiocyanates (Chirgwin et al. 1979). Two oligonucleotides corresponding to the cDNA of IGF-II were purchased from Gene-Med Biotechnologies (San Francisco, Calif., USA). The forward primer was 5'-GCTTACCGCCCCAGTGAGAC-3'; the reverse primer was 5'-TCACTTCCGATTGCTGGCCA-3'. We used initial denaturation at 94° C for 15 min with murine leukaemia virus reverse transcriptase. Amplification was then done in a DNA thermal cycler (Perkin Elmer Cetus, Takara, Japan) using an initial denaturation at 94° C for 2 min, followed by 35 cycles of denaturation at 94° C for 1 min, annealing at 55° C for 1 min, and extension at 72° C for 1 min.

Serum IGF-II molecular size was analysed by western immunoblot using specific anti-IGF-II monoclonal antibody (Amano Pharmaceutical, Nagoya, Japan). The extracted serum was electrophoresed on 16% SDS polyacrylamide gel under nonreducing conditions. The size fractionated proteins were electroblotted on the nitrocellulose sheet. The sheet was blocked with 5% weight per volume skim milk, and then incubated with anti-IGF-II monoclonal antibody for 16 h. After thorough washing, the sheet was incubated with HRP-conjugated anti-mouse IgG and then IGF-II-anti-IGF-II antibody complex were detected with enhanced chemiluminescence system (Enjoh et al. 1993).

An avidin-biotin immunoperoxidase method was used for immunohistochemistry. After paraffin sections were dewaxed by xylene and graded ethanol, the diluted mouse anti-IGF-II monoclonal antibody was mounted on the slides for 16 h at 4° C. Mouse antibody was detected with biotinylated anti-mouse Ig against goat followed by streptavidin-biotin-peroxidase (DAKO Japan, Kyoto, Japan). Peroxidase was visualized with 3.3-amino-ethylcarbazole at pH 4.9 and hydrogen peroxide.

Results

At the angulus of the stomach a 8.5×6.5 cm Borrmann's type III gastric tumour was found. Histologically, we found moderately differentiated tubular adenocarcinoma with irregular tubular patterns, and/or sheets with central necrosis of tumour cells (Fig. 1). There were massive and multi-nodular metastatic foci, up to 8.5 cm in diameter, throughout the markedly jaundiced 4082 gm liver. The tumour was far advanced, with invasion to the serosal surface and vascular involvement; there was no metastasis to lymph nodes. The histology of the metastases was similar to the gastric carcinoma. The cause of death was hepatic failure.

Immunohistochemically IGF-II was demonstrated the tumours of stomach and liver. Haematoxylin and eosin in staining of biopsied gastric carcinoma cells showed the moderately differentiated adenotubular carcinoma (Fig. 2). IGF-II was demonstrated immunohistochemically in both gastric (Fig. 3) and metastatic liver lesions (Fig. 4).

The western immunoblot analysis showed that high molecular weight IGF-II was formed mainly in tumour cells. The molecular size of the patient's IGF-II was 15 kDa (Fig. 5). RT-PCR of total RNA extracted both from gastric carcinoma and liver metastases revealed a 471 base pair (bp) DNA band shown in Fig. 6.

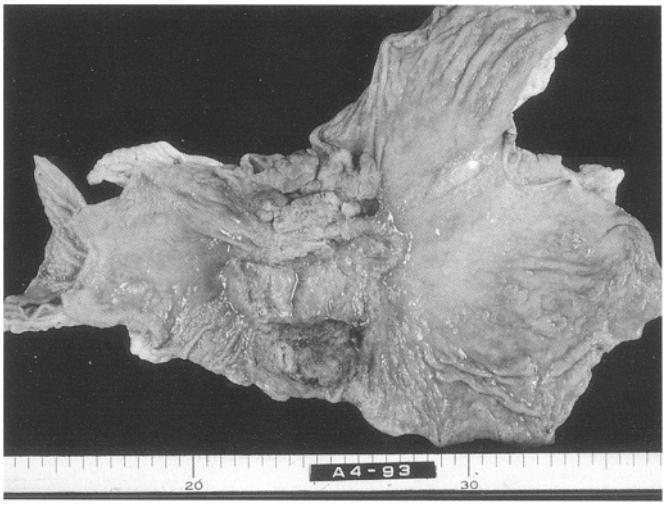


Fig. 1 Gross appearance of the gastric tumour. Note an ulcer with irregularly raised edges, consistent with Borrmann's type III cancer

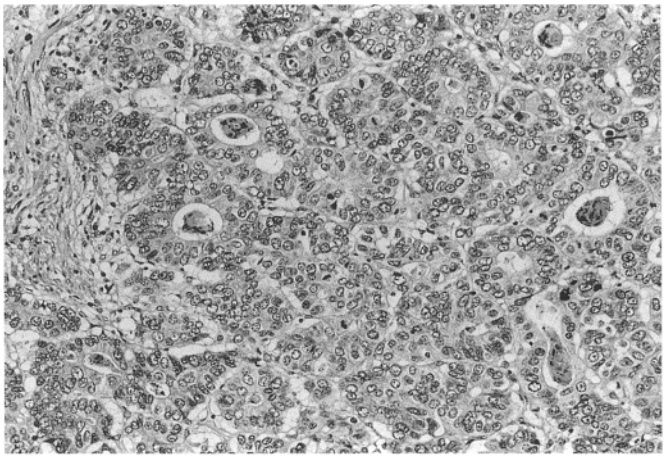


Fig. 2 Moderately differentiated tubular adenocarcinoma. Tubular arrangements with lumina predominate, with focal solid patterns. Haematoxylin and eosin, ×125

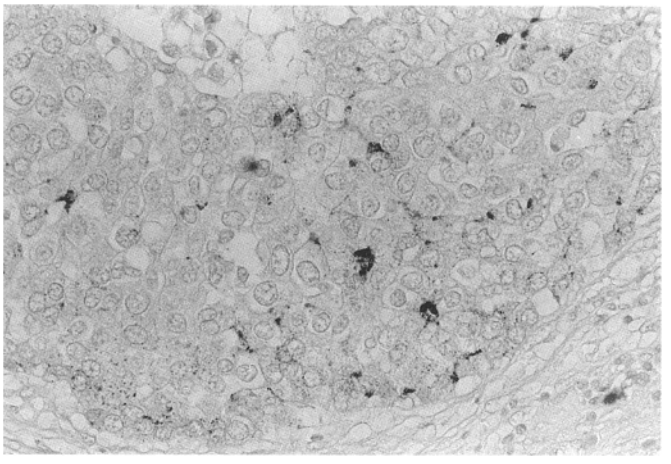


Fig. 3 Immunostain for insulin-like growth factor (IGF)-II in gastric tumour. ×250

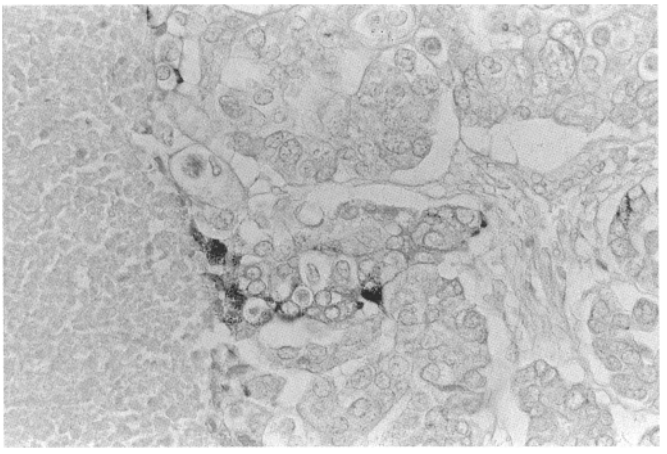


Fig. 4 IGF-II stain is also positive in metastatic tumour in the liver. Note the massive necrosis at left side. ×250

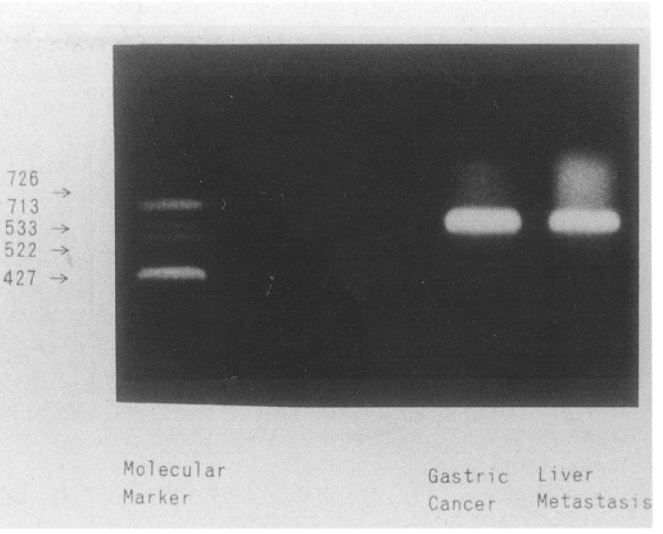


Fig. 6 Gel analysis of IGF-II cDNA. Reverse transcriptase polymerase chain reaction constructed 471 base pair size of IGF-II cDNA. From the left, the first lane shows Φx/hinfI molecular marker, the second cDNA of IGF-II from the gastric carcinoma, the third cDNA of IGF-II from metastases to liver

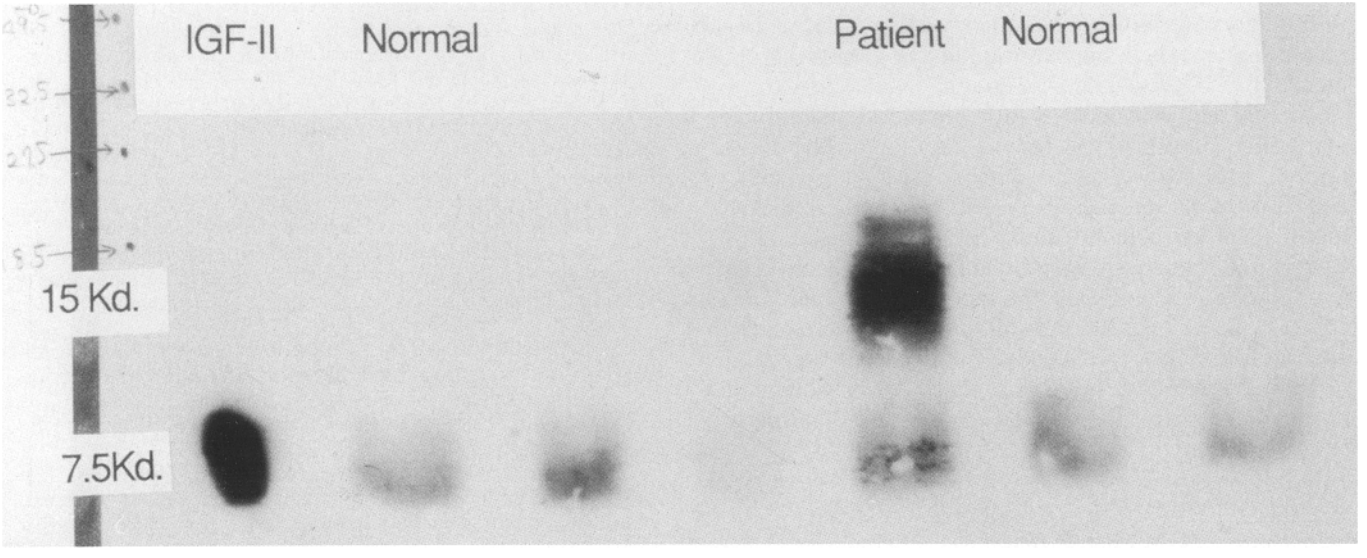
Discussion

This report presents a case of NICTH with gastric carcinoma and metastases to the liver. Insulinoma was unlikely because of low insulin level, IGF-I level and GH levels, and a slightly elevated cortisol level. CT findings first suggested a hepatoma which was consuming glucose and producing insulin-like growth factor. This possibility was subsequently eliminated and gastric carcinoma associated with hypoglycaemia was suspected.

Two groups have recently developed specific antibody for big IGF-II and 7.5 kDa Mr IGF-II. One developed a rabbit polyclonal antiserum directed against the first 21 amino acids of pro-IGF-II (Daughaday and Trivedi 1992). The other developed monoclonal antibody against recombinant human IGF-II (Enjoh et al. 1993). The polyclonal antibody detects pro-IGF-II, or big IGF-II, in NICTH; the monoclonal antibody can specifically detect 7.5 kDa Mr of IGF-II of NICTH. The abnormal functioning of NICTH tumours can be monitored with these two specific antibodies. Immunostaining, using the monoclonal antibody in a peroxidase reaction, demonstrated the presence of IGF-II peptide in both stomach and liver tumour cells; normal cells were not stained.

In our study we synthesized two primers which correspond to pre-pro-IGF-II cDNA. These two oligonucleotides constructed 471 bp cDNA, corresponding to 157 amino acids of alanine and lysine at RT-PCR, suggesting that tumour cells transcribe the mRNA of pro-IGF-II and synthesize it. IGF-II mRNA expression has been studied in various tumours (Gary et al. 1987). In most cases, the

Fig. 5 Western blot of patient serum. The serum from the patient demonstrated two forms of IGF-II, 15 kDa and 7.5 kDa. In contrast, normal serum showed one form IGF-II, 7.5 kDa



mRNA size were 4.5 kilobases (kb) and 5.4 kb; however, some tumours expressed only the 5.4 kb type while hepatoma showed additional bands. We examined the coding region with RT-PCR, only; further tissue specificity study with the northern blot technique is necessary.

Our western blot results, demonstrating 15 kDa Mr IGF-II, were compatible with a previous report which used specific monoclonal antibody (Enjoh et al. 1993). However, it has not been clearly established which size of IGF-II interacts directly with the target cell receptor. Pro-IGF-II is usually cleaved before the arginine position, in the cell, and is secreted into the systemic flow in its active form (Bell et al. 1984). The processing of propeptide is often incomplete in NICTH patients tumours, giving an elevated level of immunoreactive factors (Haselbacher et al. 1987).

The affinity to IGF-II receptor of 7.5 kDa IGF-II and 15 kDa IGF-II is similar (Gowan et al. 1987). In contrast the affinity of big IGF-II and authentic IGF-II for insulin receptors may differ (Zapf et al. 1992). More recently, Hari et al. (1987) demonstrated that an antibody to the IGF-II receptor stimulated glycogen synthesis in hepatoma cells. In human skin fibroblast, the stimulating effect of IGF on glucose transport and metabolism cannot be explained by a cross reaction in the insulin receptor (Rechler and Nissley 1985). The action of IGF-II to cause hypoglycaemia might be mediated through both insulin receptor and IGF receptor.

The exact process by which big IGF-II induces hypoglycaemia in NICTH, with normal IGF-II also present, is not yet understood. Zapf et al. (1990) proposed that the increased bioavailability of big IGF-II complex, together with IGF-II binding protein-2 (IGFBP-2), due to unrestricted capillary passage and the enhanced insulin bioactivity of big IGF-II pool, result in an increased insulin-like potential in IGF-II. Thus IGFBP appears to be the key to clarifying the mechanism of IGF-II induced hypoglycaemia. The distribution of big IGF-II between 150 kDa and 50 kDa IGFBP complex and their availability may also be significant factors (Zapf et al. 1992). We may speculate that free IGF-II may be active, while IGF-II coupled with IGFBP might be inactive in terms of the insulin receptors, in consuming plasma glucose in target tissues.

This is the first report of immunohistochemically-proven IGF-II production in gastric carcinoma. The primary tumour and its metastases expressed pro-IGF-II mRNA and 15 kDa Mr of IGF-II as demonstrated by the anti-monoclonal antibody western blot method. Although big IGF-II may be crucial in the pathogenesis of NICTH, it is not the only hypoglycaemic factor; insulin and IGF receptors, as well as IGFBP distribution may also be involved. Further study is needed to pursue the mechanism of hypoglycaemia of NICTH in terms of a binding study of insulin receptor, IGF receptor and IGF-II binding protein.

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