Table 1. Relationship between sialyl-Tn immunoreactivity and several pathological parameters (depth of penetration of gastric wall, lymph node metastases and venous invasion)

	Negative (n = 25)	≤ 5% (n = 10)	5–50% (n = 51)	≥ 50% (n = 14)	P value
Serosal invasion (n = 74)	22(88.0%)	4(40.0%)	37(72.6%)	11(78.6%)	0.04
Lymph node metastases (n = 72)	17(68.0%)	6(60.0%)	37(72.6%)	12(85.7%)	0.53
Venous invasion (n = 55)	14(56.0%)	3(30.0%)	28(54.9%)	10(71.4%)	0.26

The first concerns the difficulty of interpreting the meaning of the numerous negative cases from a pragmatic standpoint. In fact, 50% of the negative cases of Ma's series [1] displayed invasion into lymphatics and 38% were in stages III or IV. In our series [3], most of the negative cases also displayed features of aggressiveness such as serosal invasion (88.0%), venous invasion (56.0%), lymph vessel permeation (84.0%) and lymph node metastases (68.0%) (Table 1).

The second point concerns the putative relationship between sialyl-Tn expression and the histological type of gastric carcinoma. In contrast to Ma and colleagues [1], we found (data not previously shown) a significant relationship between sialyl-Tn expression and histological type, according to Laurén's classification [5]: intestinal 62.7%, diffuse 25.3% and unclassifiable 12.0% (P = 0.02). We cannot compare this finding with those of Ma's study [1] because these authors have not used Laurén's classification in their study. However, the recent finding of Iwata and associates [6], showing that sialyl-Tn is a marker of goblet cells, partly supports the assumption that sialyl-Tn expression is related to cell differentiation.

Taking Ma's results [1] together with our own [2, 3] and those from other groups [4], we conclude that the practical usefulness of sialyl-Tn expression as a marker of biological aggressiveness and/or prognosis in gastric cancer still remains to be elucidated.

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An Erythropoietin-producing Endometrium Carcinoma

J. Asin, H.P. Muller and R. Fijnheer

ERYTHROPOIETIN IS a glycoprotein with a molecular weight of appoximately 32–40 kDa. It is a hormone involved in the production of erythrocytes, produced in the kidneys and in small amounts elsewhere, mainly in the liver. The production is stimulated when the oxygenation of the tissues is insufficient. This can be the result of a decreased quality or quantity of the erythrocytes, or hypoxaemia [1–3]. Erythropoietin controls differentation of precursor cells into erythroid lineage, rapidly induces RNA synthesis, and appears to influence the release of marrow reticulocytes [4].

Marked erythrocytosis and high serum levels of erythropoietin have also been reported in case of renal cell carcinoma, renal artery stenosis, cystic kidneys, hepatoma, haemangiosarcoma, leiomyoma, pheochromocytoma, androgen-producing ovarium tumours and Cushing's disease [3, 5]. There has never been a report of marked erythrocytosis and elevated serum erythropoietin levels in case of endometrial carcinoma. Here we report a case of an erythropoietin-producing endometrium carcinoma.

A 60-year-old woman was seen because of postmenopausal bleeding in April 1992. Histological examination showed a moderately differentiated adenocarcinoma of the endometrium. Laboratory data revealed an erythrocyte count of 4.81 × 10E12/1 [normal range (N)] = 3.69–4.88 × 10E12/1], Hb 8.1 mmol/l (N = 7.5–9.6 mmol/l) and increased serum CA-125 (170 kU/l, N = < 20 kU/l). She underwent a laparatomy, and unexpectedly the peritoneum, omentum, diaphragm, and ovaria were

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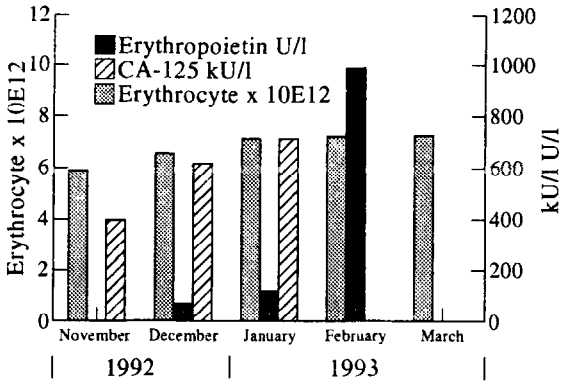

Figure 1. Correlation between erythrocyte count, serum CA-125 and erythropoietin in time. The first erythropoietin measurement is a serum level, the other two data are measurements of ascites fluid.

full of metastatic tumour. Para-aortal lymph glands were enlarged. it was decided to perform a total uterus extirpation with omentum resection, because an ovarium carcinoma could not be excluded with certainty. Histologically, the uterus showed an extensive, poorly differentiated adenosquamous endometrium carcinoma extending into the cervix and myometrium, and metastasis to ovaria, peritoneum and omentum.

Hormonal therapy was refused by the patient. In November 1992, a haematological examination showed a marked erythrocytosis (5.97 × 10E12/l; Hb 10.4 mmol/l), which increased in the following months (December 1992 6.65 × 10E12/l, Hb 10.9 mmol/l; January 1993 7.19 × 10E12/l, Hb 11.2 mmol/l; February 1993 7.23 10E12/l, Hb 11.0 mmol/l; March 1993 7.29 × 10E12, Hb 11.4 mmol/l) (Figure 1). Simultaneously, the serum CA-125 increased (November 1992 399 kU/l, December 1992 619 kU/l, January 1993 718 kU/l) (Figure 1) and an elevated serum erythropoietin level was found (January 1993 66 U/l, N = 8–34 U/l) (Figure 1). In January 1993, ascites appeared with metastatic tumour cells comparable to the adenosquamous endometrium carcinoma of the uterus. Marked pathological lymph glands para aortal were found at computed tomography. Liver, kidneys, spleen and pelvis showed no abnormity. There were no signs of heart or respiratory failure.

In the following months, large amounts of ascites had to be removed. The erythropoietin level of the ascites fluid was examined twice and found to be raised (February 1993 114 U/l and March 996 U/l N = 8–34). In February 1993, a course of hormonal treatment with medroxyprogesterone was started without success. The patient died in March 1993. Permission for postmortem examination was not obtained.

In this patient, there appeared to be a correlation between serum CA-125, the amount of ascites produced and the erythropoietin levels in serum and ascites. There was no evidence for the site of erythropoietin production. The patient had neither signs of renal cell carcinoma, nor evidence of another cause of increased erythropoietin production and sectetion. Therefore, it is assumed that the increased serum and ascites fluid erythropoietin levels were the result of secretion of erythropoietin by adenosquamous endometrium carcinoma cells.

Erythropoietin measurement is an important step in the differential diagnosis of erythrocytosis [2, 6], and should be performed in case of unexplained high erythrocyte counts.

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Adhesion to Type V Collagen and Cloning Efficiency in Agar of 8701-BC Breast Cancer Cells

C. Luparello

IN AN earlier paper published in this journal [1], we described the restraining effect of type V collagen on the adhesion and proliferation of a neoplastic cell line (8701-BC), derived from a primary ductal infiltrating carcinoma (DIC) of the breast [2], which is characterised by some phenotypical heterogeneity [3,4] and a differential response to various collagen types when used as culture substrates [5–8]. Recently, we have determined the cloning efficiency (CE) of 8701-BC cells by seeding in agar at different concentrations [4], i.e. 0.3 and 0.6%, the latter being a selective support for the clonogenic growth of those 8701-BC cell subpopulation(s) endowed with enhanced proliferative rate and chemoinvasive ability [4,9] and thus regarded as more aggressive *in vitro*.

The occurrence of a defective adhesive capacity (approximately 40%) of 8701-BC cells onto type V collagen substrate [1] prompted us to investigate if the cell subgroup which recognised this substrate for attachment showed a different CE value in 0.6% agar and, therefore, different malignant properties *in vitro*, from that of cells unable to adhere onto it. For this purpose, 35 mm diameter Falcon dishes (Beckton Dickinson, Lincoln Park, New Jersey, U.S.A.) were coated with 100 μg of type V collagen (Sigma, St Louis, Missouri, U.S.A.) as already reported [1,6], and 8701-BC cells at passages 64–70 were plated at a concentration of 3 × 10^4/dish in serum-free RPMI medium and allowed to settle for 24 h. Floating (V−) and attached (V+) cells were then harvested separately, the latter by EDTA-trypsin treatment, and after cell viability was checked by Trypan blue staining, cells were plated in 0.6% agar

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