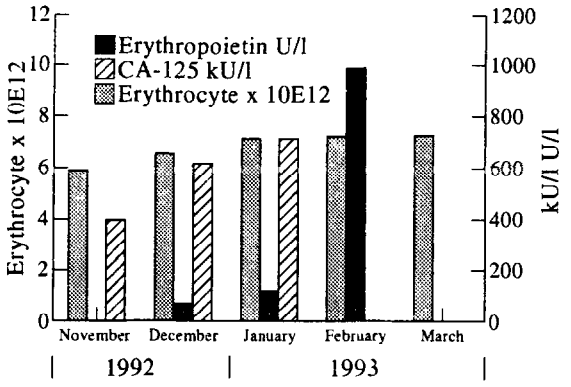

Figure 1. Correlation between erythrocyte count, serum CA-125 and erythropoietin in time. The first erythropoietin measurement is a serum level, the other two data are measurements of ascites fluid.

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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Table 1. Cloning efficiency (CE) of 8701-BC cells in 0.6% agar after selection by adhesion onto type V collagen substrates

Cell subpopulation	Cell viability (%)	No. of assays	Mean CE
	100	16	0.044*
V+	100	20	0.030
V−	27	20	0.112

*CE of the parental 8701-BC line [4].

at a concentration of 2000 viable cells/dish and incubated at 37°C in a 5% CO_2 atmosphere for the appropriate time, after which CE values were calculated by the formula:

$$CE = \text{colony number/initial cell concentration} \times 100,$$

as described elsewhere [4].

Table 1 reports the viability and CE values of 8701-BC cells selected in the presence of type V collagen, compared with previous results obtained with the parental cell line [4]. Approximately 73% of V− cells clearly undergo cell death after 24-h incubation, as revealed by the lowering of Trypan blue exclusion after staining. Interestingly, the surviving fraction of the V− cell subpopulation, once allowed to grow in 0.6% agar, shows a more than 3-fold increase of clonal proliferation versus V+ cells. It is noteworthy also that the CE of the unselected 8701-BC line is lower, although to a minor extent, than that of V− cells. Consequently, the data obtained indicate that type V collagen exerts a clonal selection on the heterogeneous 8701-BC cell line, allowing the adhesion and survival of the potentially less malignant cells (by involvement of the 67 kDa [10] and/or other surface receptors), and inhibiting the propagation of cells endowed with a stronger neoplastic aggressiveness.

It is well known that in development and cancer, the extracellular matrix (ECM) undergoes massive compositional changes, both quantitative and qualitative which, in turn, influence the state of cell differentiation by modifying the multisignal network of interactions. We have reported previously that individual collagen species are able to elicit diverse, and in some cases opposite, responses by DIC cells *in vitro*. In particular, type V collagen was found to be an anti-adhesive (in part), antiproliferative and anti-locomotory substrate, even when used as hybrid matrices with type I collagen [1,7,8]. A similar inhibitory effect by this collagen type was also proven in other cell systems, both normal and transformed ([11] and references therein). The data here reported show another interesting "anti-cancer" property of collagen type V, at least for the cell line under study.

Moreover, the present results give further support to the hypothesis that the over-deposition of type V collagen occurring in the stroma of DIC [12,13] may be regarded as a true defensive host reaction. This could negatively regulate the progression of DIC *in vivo*, being one of the "instructive" ECM signals which concomitantly contributes to directing the tumour cell population towards different levels of malignancy by modulating gene expression and phenotypical selection.

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Intracranial Germ Cell Tumours Presenting With Hypopituitarism. Successful Treatment with Chemotherapy Alone

J.A. Bridgewater, R.L. Souhami, J. Allgrove, B. Kendall and J. Pritchard

INTRACRANIAL GERM cell tumours (GCT) represent only 0.3–3.4% of all primary intracranial tumours in children, and more than 95% of them present with the consequences of a mass lesion in either suprasellar or pineal regions. Pineal tumours

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