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Localisation of CEA, β-HCG, SP1, and keratin in the tissue of lung carcinomas

An immunohistochemical study

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Summary. One hundred and twenty seven cases of lung tumors were studied by the immunoperoxidase technique for the presence of CEA and β-HCG. Twenty-nine of these tumors were additionally stained for keratin and SP1. CEA and SP1 could be demonstrated in 80% of the studied cases, while β-HCG was found in only 9%. SP1 revealed an almost identical staining pattern to CEA and keratin was found only in squamous cell carcinomas. The tissue positivity of none of these three markers correlated with tumor size, lymphnodal involvement or histological type.

Key words: CEA – β-HCG – SP1 – Keratin – Lung tumors.

Introduction

Since the discovery of carcinoembryonic antigen (CEA) in colonic tumor patients' sera, by Thomson et al. (1969), the antigen has also been detected in the sera of patients with non-gastro-intestinal tract tumors, including lung tumors (Vincent et al. 1975). Human chorionic gonadotropin (HCG) and pregnancy specific-β_1 glycoprotein (SP1) (Bohn 1971) which are normally produced by the placenta, have been demonstrated in the sera of patients with trophoblastic (Seppälä et al. 1978) and non-trophoblastic tumors (Würz et al. 1979; Searle et al. 1978; Braunstein et al. 1973). Utilising the immunoperoxidase technique, CEA and β-HCG have also been demonstrated in the tissues of lung tumors. However, there are varying results concerning CEA tissue positivity, the incidence was reported to be highest

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in adenocarcinomas and lowest in anaplastic carcinomas (Pascal et al. 1977; Ford et al. 1981). The results are difficult to compare, because different classifications and different interpretation criteria of CEA positivity in keratinizing areas of squamous cell carcinomas have been used.

To date, most authors who have demonstrated HCG and CEA in lung carcinomas have only stated the frequency of positivity of these markers, without giving a comparative description of their localisation in the tumor tissues or cells. The aim of this study, therefore, was to compare CEA, SP1, and β-HCG positivity in the lung tumor tissue in relation to tumor stage and histological classification, and to establish the site of production of these antigens by comparing their staining pattern. Additionally, an anti-keratin-antiserum was used to classify the tumors better and to compare keratin and CEA staining.

Material and methods

Tumors. Tissue samples of 127 lung carcinomas were obtained either surgically or by biopsy from patients treated at the "Robert-Koch-Hospital" of the University of Freiburg i. Br. over the last few years. The formalin-fixed and paraffin embedded tissues (obtained from the Institute of Pathology of the University) were sectioned at 5 μm.

The histological diagnosis of the tumors was made independently by two of the investigators on HE, PAS, and EvG stained sections. The classification according to the World Health Organization (WHO 1981) was as shown in Table 1.

Antisera. All tumors were stained for CEA and β-HCG, while only 29 of them were stained additionally with SP1- and keratin-antisera and monoclonal CEA antibodies (Mab). The polyclonal CEA-, β-HCG-, and SP1-antisera and the peroxidase labelled anti-rabbit serum were purchased from Dakopatts (Denmark), the keratin-antiserum from Immulok Inc., California (USA). The monoclonal CEA-antiserum was produced in mice by our own group as described previously (Grunert et al. 1982).

Immunohistochemical techniques. The tissue sections were deparaffinized and endogenous peroxidase activity was blocked with 1% hydrogen peroxidase in absolute methanol (30 min). To reduce background staining, the sections were incubated for 30 min with 5% ovalbumin. Between each incubation step the sections were washed with Tris buffered saline, pH 7.4. The incubation with the first antiserum was performed for one hour, and with the second peroxidase labelled antiserum for 30 min at room temperature. Peroxidase activity was developed by DAB with 0.012% hydrogen peroxide. The sections were counterstained in Mayer's haemalaun, dehydrated and mounted in Entellan. The commercial CEA-antiserum had to be absorbed with a normal lung extract to prevent cross-reaction with NCA (von Kleist, S. et al. 1972). The other antisera were used unabsorbed, all at a dilution of 1/30, except for the Mab-anti-

Table 1. Histological classification of the cases studied

Diagnosis	No. of cases
Squamous cell carcinoma	51
Small cell carcinoma	37
Adenocarcinoma	25
Large cell carcinoma	11
Adenosquamous carcinoma	2
Carcinoid	1

CEA. In addition to the staining with the polyclonal anti-CEA serum, 29 sections were incubated with the monoclonal antiserum directed against one CEA specific determinant.

In order to check for a possible interference between the CEA-antiserum and keratin, sections were first incubated with a keratin-antiserum raised in rabbits, and then with the monoclonal CEA antibody. If there was any interference between keratin and CEA-antiserum, CEA staining should be inhibited.

Sections of human colon tumors served as positive controls for CEA staining and sections of human placenta for both β-HCG and SP1. The keratin positivity was controlled by comparing it with the keratinizing areas of the HE stained sections in serial slides. Sections of each tumor which were incubated with the homologous normal serum instead of the specific antiserum, served as negative controls for all markers. Furthermore, the absorption of the CEA-antiserum with CEA (prepared from liver metastases of a colon carcinoma), of the SP1-antiserum with SP1 (kindly provided by Dr. Bohn, Behringwerke), and of the β-HCG-antiserum with β-HCG (Boehringer, Mannheim, FRG) abolished positive staining of the control sections.

Results

In the lung tumor tissues studied, CEA and SP1 were demonstrated in 80%, while β-HCG was found in only 9%. SP1 revealed an almost identical staining pattern to that of CEA in the 29 tumors studied, and of the 29 tumors stained with anti-keratin-serum, only the 14 squamous cell carcinomas were positive. The tissue positivity of none of the three markers correlated with tumor size, lymph-nodal involvement or histological type.

CEA

If the different histological tumor types were analyzed, the highest incidence of positive CEA staining was seen in adenocarcinomas (100%), in which the staining intensity was also highest.

CEA could be detected in almost every tumor cell with an intense positivity at the luminal border of highly differentiated bronchoalveolar carcinomas, and a diffuse intracytoplasmic staining was seen in moderately or poorly differentiated carcinomas.

In squamous cell carcinomas the stain was mainly restricted to the centers of the epidermoid cell nests, which often showed keratinization. We could also label these keratinizing areas with an anti-keratin-serum and found a similar staining pattern to CEA. But CEA was also detected in cells surrounding these areas and even in some squamous cell carcinomas which were not keratinized. However, in these few cases the pattern was different, with more intense intracellular staining and a larger part of the tumor staining positive (Fig. 1 and 2).

In the 14 squamous carcinoma cases pre-incubated with an antikeratin-antiserum, and then incubated with a monoclonal anti-CEA-antiserum, the staining intensity of CEA was not diminished.

In large cell carcinomas CEA could be detected with the same frequency as in squamous cell carcinomas (91%, 10/11 cases). In all these tumors the CEA location was intracellular with many of the cells showing a positive stain along the whole outer cell border. Small cell carcinomas were only in 48% positive with no characteristic staining pattern.

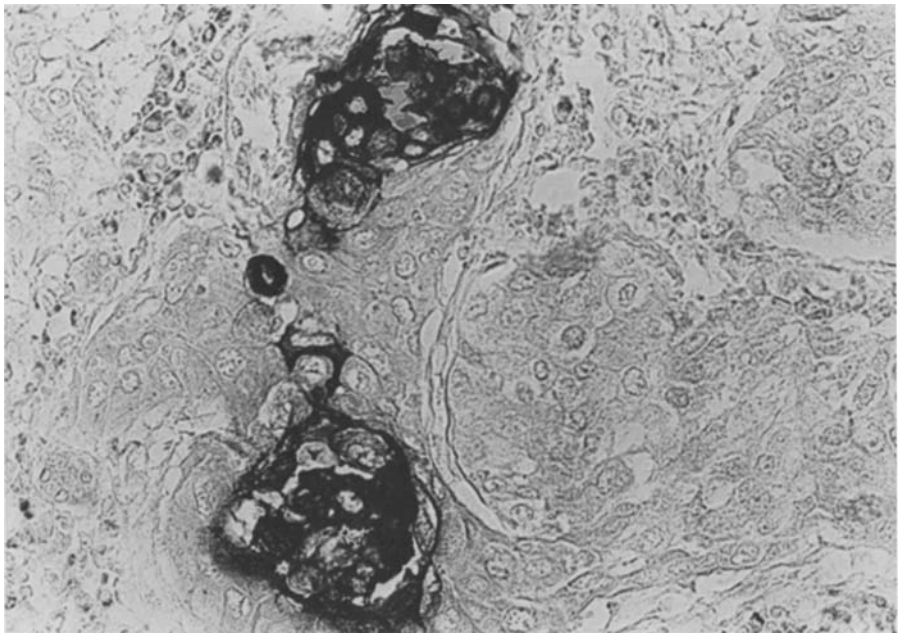

Fig. 1. CEA staining in keratinizing areas of a squamous cell carcinoma. No counterstaining $\times 400$

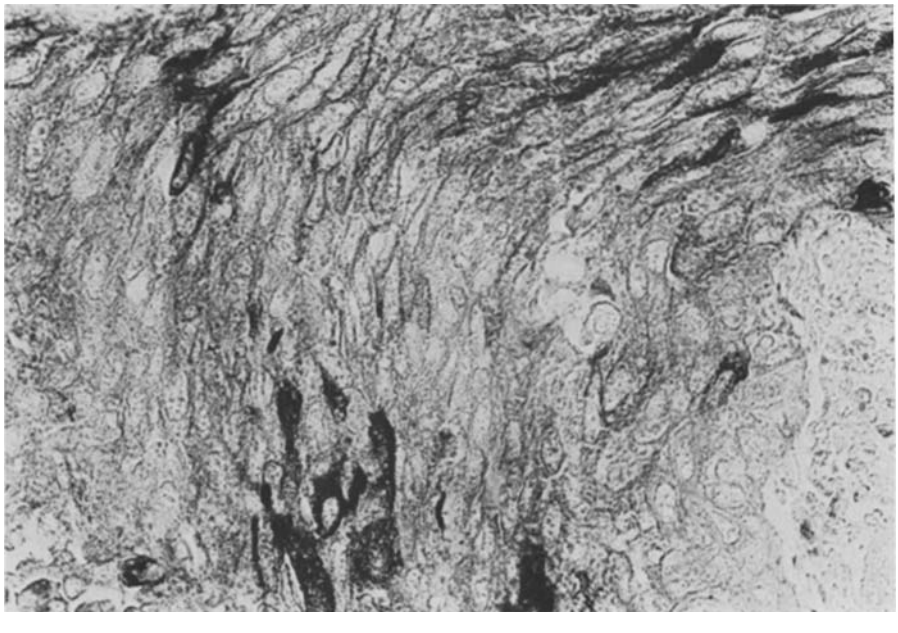

Fig. 2. CEA staining in a non-keratinizing area of a squamous cell carcinoma (another carcinoma as Fig. 1). No counterstaining $\times 400$

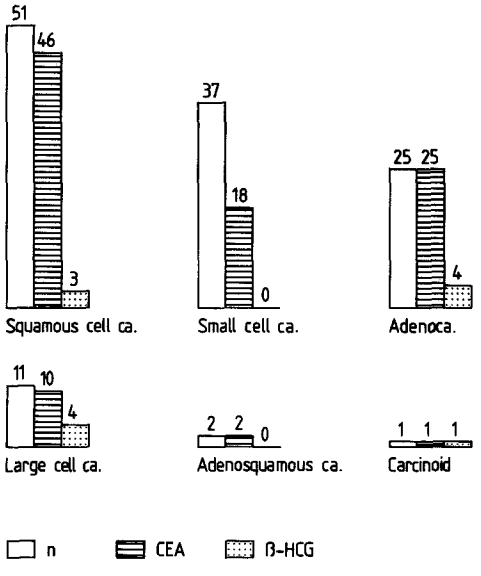

Fig. 3. Frequency of CEA and β-HCG in different lung carcinomas (n = total number of cases)

HCG

HCG was detected in only 12 of the 127 tumors. Interestingly, it never appeared in small cell carcinomas. The highest incidence was seen in large cell carcinomas (36%) (Fig. 3). In these 12 positive cases. 4 characteristic staining patterns were revealed:

– 3 tumors (No. 1–3, Table 2), all of different histological types, showed β-HCG intracytoplasmatically in spindle-form near the nucleus (Fig. 4). Most cells of these tumors were β-HCG positive and in the same cells CEA could also be detected with its characteristic staining pattern.

– In 3 further carcinomas (No. 4–6, Table 2), β-HCG was found in fine granular deposits throughout the whole cytoplasm of some undifferentiated cells being arranged in clusters. They were, however, not stainable for CEA, and had no contact with the CEA positive differentiated cell formations (Fig. 5).

– In a third group of tumors (No. 7–9, Table 2), up to 80% of all cells were positive for CEA, while β-HCG was located in only a few single cells (~1%) spread throughout the tumor. These cells were in differentiated formations, i.e. squamous or adenoid structures, and did not differ morphologically from the main bulk of neighbouring cells.

– In 2 other tumors (No. 10, 11, Table 2), β-HCG could be found in a group of undifferentiated cells, which also stained positive for CEA. However, these cell formations were only a small part of the CEA positive staining tumor cells.

The only carcinoid studied showed up to 70% β-HCG positive cells, while CEA was seen in only 10% of the tumor in different cells (Table 2).

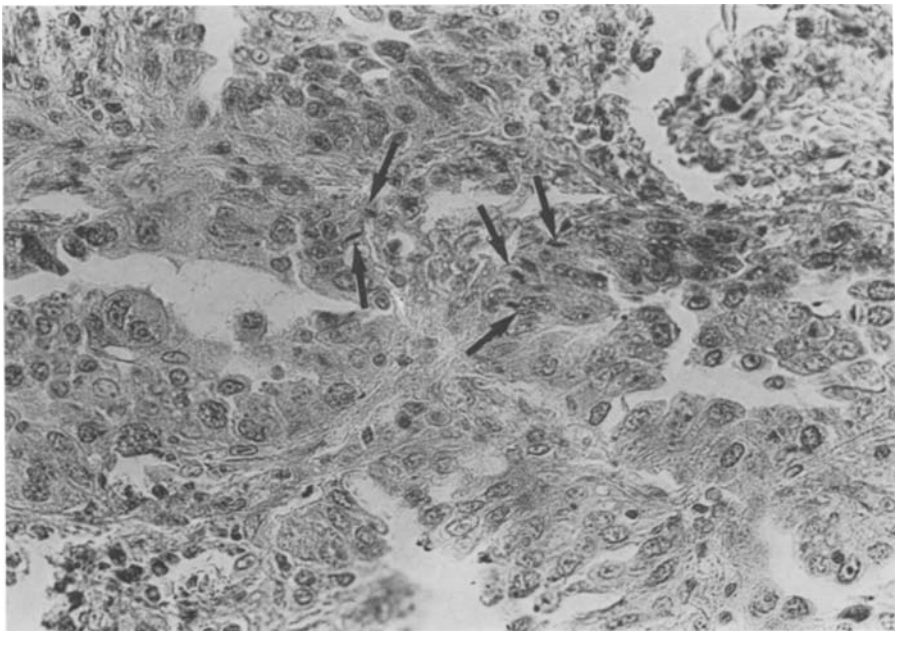

Fig. 4. Spindle-shaped β-HCG in cells of an adenocarcinoma. HE counterstaining $\times 400$

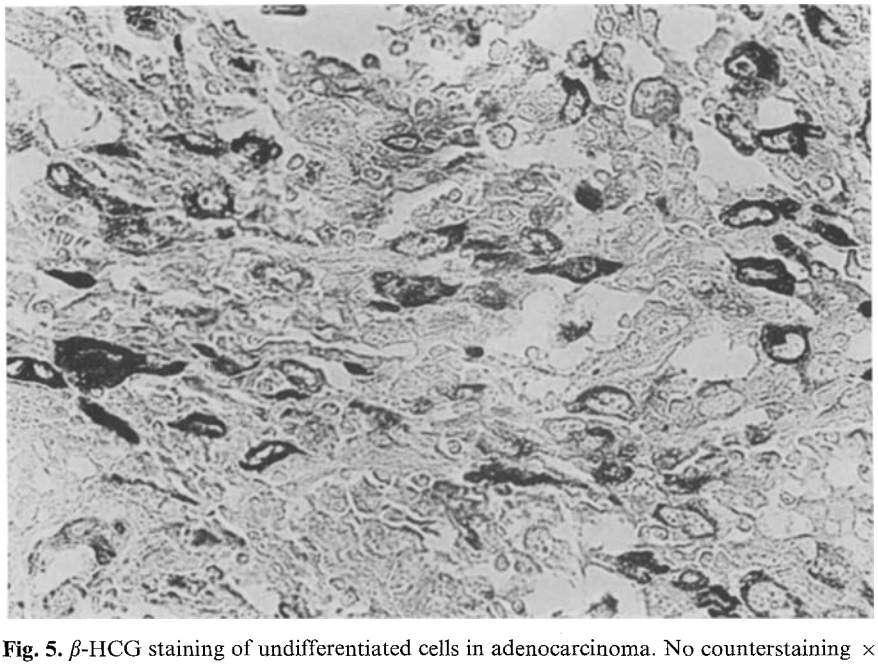

Fig. 5. β-HCG staining of undifferentiated cells in adenocarcinoma. No counterstaining $\times 500$

Table 2. Characteristics of β-HCG positive lung tumors

No.	AGE/years	m/f	TNM-Stage	Histological Type
1	64	m	$T_1N_oM_o$	Large cell carcinoma
2	56	m	$T_3N_1M_x$	Adenocarcinoma
3	65	m	$T_3N_2M_x$	Squamous cell carcinoma
4	56	m	$T_2N_oM_o$	Large cell carcinoma
5	50	f	$T_3N_1M_x$	Large cell carcinoma
6	66	f	$T_xN_xM_x$	Adenocarcinoma
7	63	m	$T_2N_oM_o$	Adenocarcinoma
8	51	m	$T_2N_oM_o$	Squamous cell carcinoma
9	75	m	$T_xN_xM_x$	Adenocarcinoma
10	58	m	$T_3N_2M_x$	Squamous cell carcinoma
11	58	f	$T_2N_oM_o$	Large cell carcinoma
12	54	m	$T_2N_1M_o$	Carcinoid tumor

Discussion

In the 127 lung tumors studied CEA was detected with a high incidence in adenocarcinomas (100%), in squamous cell carcinomas (90%), and in large cell carcinomas (91%).

As CEA staining in squamous cell carcinomas was often restricted to the epidermoid cell nests and almost identical to the keratin staining, we preincubated sections of 14 squamous cell carcinomas with a polyclonal anti-keratin serum and then we stained them with the monoclonal CEA antibody. The staining intensity of CEA was not diminished. Therefore we consider that there is no interference between CEA and keratin, but a parallel expression of these two substances in squamous cell carcinomas. Hence we cannot confirm the suggestion of Pascal et al. (1977) that CEA positivity in keratinizing areas of squamous cell carcinomas is due to cross-reactivity of CEA with keratin or a keratin precursor. The CEA staining pattern of the adenocarcinomas with the intracytoplasmic and luminal border staining was similar to that described by Hill et al. (1979). With regard to the high overall incidence of CEA positivity (i.e. 80%) our results are in good correlation with those of Ford et al. (1981) and Gropp et al. (1981).

The same staining pattern was also seen with SP1 staining. However, the staining intensity of SP1 was weaker and the background staining higher. We believe that additional staining of lung tumors with SP1 antiserum is not advantageous.

Although SP1 and β-HCG are normally produced by the same cells of the placenta, in lung tumors they show a different staining pattern, β-HCG sometimes being produced in totally different cells.

HCG was localized immunohistologically by the immunofluorescence technique in lung tumor tissue for the first time by Becker et al. (1968) and Cottrell et al. (1968). However, the reacting cell was not identified.

Furthermore, reports concerning β-HCG in lung cancers differ widely: while Nishiyama et al. (1980) reported 20% positive lung tumors (7/35) of different histological types, Wilson et al. (1981) demonstrated β-HCG production in 84% of the 61 tumors studied. In comparison with these reports, the incidence of our β-HCG positive cases was rather low (12/127), i.e. 9%. Only the great incidence of β-HCG in large cell carcinomas could be confirmed (36%). While each histological type has its own characteristic CEA staining pattern, for β-HCG there were four different staining patterns, independent of the histological diagnosis. In contrast to Gropp et al. (1981) we could not detect β-HCG in small cell carcinomas.

In our study all tumors with β-HCG positivity were also stainable for CEA, but not always in the same cells. In some tumors there are clusters of inconspicuous undifferentiated tumor cells, producing only β-HCG. These cells are small and do not resemble the syncitiotrophoblastic-like-giant cells of germ cells tumors.

Tissue positivity of none of the three markers correlated with tumor size and lymph-nodal involvement. Further studies will show whether CEA together with β-HCG can act as markers with prognostic significance.

References

Becker KL, Cottrell JC, Moore CF, Winnacker JL, Matthews MJ, Katz S (1968) Endocrine studies in a patient with gonadotropin secreting bronchiogenic carcinoma. J Clin Endocrinol Metab 28:809–812

Bohn H (1971) Nachweis und Charakterisierung von Schwangerschaftsproteinen in der menschlichen Plazenta, sowie ihre quantitative immunologische Bestimmung im Serum von schwangeren Frauen. Arch Gynaekol 210:440–457

Braunstein GD, Vaitukaitis JL, Carbone PP, Ross GT (1973) Ectopic production of human chorionic gonadotropin by neoplasms. Ann Int Med 78:39–45

Cottrell JC, Becker KL, Moore CF (1968) Immunofluorescent studies in gonadotropin secreting bronchiogenic carcinomas. Am J Clin, Pathol 50:422–430

Ford CJH, Stokes HJ, Newmann CE (1981) Carcinoembryonic antigen and prognosis after medical surgery for lung cancer: Immunocytochemical localisation and serum levels. Br J Cancer 44:145–153

Gropp C, Sostman H, Luster W, Kalbfleisch H, Lehmann FG, Havemann K (1981) ACTH, β-lipotrophin, β-endorphin, β-HCG, calcitonin, and CEA in lung tumor tissue: In: Uhlenbruck G, Wintzer G (eds) CEA und andere Tumormarker. Tumordiagnostik Verlag pp 217–226

Grunert F, Wank K, Luckenbach GA, von Kleist S (1982) Monoclonal antibodies against CEA: Comparison of the immunoprecipitates by fingerprint analysis. Oncodev Biol and Med 3:191–200

Hill TA, McDowell EM, and Trump BF (1979) Carcinoembryonic antigen (CEA) in normal pre-malignant and malignant lung tissue. Carcinoembryonic proteins, vol II Lehmann FG, (ed) Elsevier/Holland and Biochemical Press: 163–168

von Kleist S, Chavanel G, Burtin P et al. (1972) Identification of normal antigen that cross-reacts with the carcinoembryonic antigen. Proc Natl Acad Sci USA 69:2492–2494

Nishiyama T, Stolbach LL, Rule AH, DeLellis R, Inglis NR, Fishman WH (1980) Expression of oncodevelopental markers (Regan isozyme, β-HCG, CEA) in tumor tissues and uninvolved bronchial mucosa. An Immunohistochemical study. Acta Histochem Cytochem 13:245–253

Pascal RR, Mesa-Tejada R, Bennett SJ, Garcea A, Fenoglio CM (1977) Carcinoembryonic antigen: Immunohistologic identification in invasive and intra-epithelial carcinomas of the lung. Arch Pathol Lab Med 101:568–571

Searle F, Bagshawe KD, Leake BA, Dent J (1978) Serum SP1 pregnancy-specific β-glycoprotein in choriocarcinoma and other neoplastic disease. Lancet II, 579

Seppälä M, Rutanen E-M, Heikinheim M, Jalanko H, Engvall E (1978) Detection of trophoblastic tumor activity by pregnancy-specific β-1-glycoprotein. Int J Cancer 21:265–267

Thomson DMP, Krupey J, Freedman SO, Gold P (1969) The radioimmunoassay of circulating carcinoembryonic antigen of the human digestive system. Proc Natl Acad Sci USA 64:161–167

Vincent RG, Chu TM, Fergen TB, Ostrander M (1975) Carcinoembryonic antigen in 228 patients with carcinoma of the lung. Cancer 36:2069–2076

Wilson TS, McDowell EM, McIntire KR, Trump BF (1981) Elaboration of human chorionic gonadotropin by lung tumors. Arch Pathol Lab Med 105:169–173

Würz H, Geiger W, Grah H, Hoffmann M (1979) Simultaneous assays for SP1, SP3, CEA, AFP, and β-HCG in the serum of patients with breast cancer and other non-trophoblastic malignancies. Carcinoembryonic proteins, vol II. Lehmann FG (ed), Elsevier/Holland and Biomedical Press 487–490

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