DNA PLOIDY IN THYMOMA AND ASSOCIATED MULTIPLE PRIMARY MALIGNANCIES IN THE SAME PATIENT

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SUMMARY – Thymoma is an uncommon neoplasm derived from thymic epithelium. It is located in the anterosuperior mediastinum. Thymoma may be associated with different types of additional primary malignancies; however, colorectal adenocarcinoma and thyroid cancer appear to be most common. A case is presented of a 63-year-old woman with type A thymoma and two other primary carcinomas of the breast and colon that were previously diagnosed. The patient underwent surgery due to metastatic colon cancer of the lung and yet another primary tumor was found in the mediastinum, later diagnosed as thymoma. A normal diploid pattern was found in the samples of thymoma and colon carcinoma, whereas those of breast carcinoma and metastatic tumor of the lung showed aneuploidy. On the basis of previous studies and our case, it is concluded that the occurrence of extrathymic malignancies does not correlate with the biologic behavior and DNA ploidy of thymoma.

Key words: *Thymus neoplasms – diagnosis; Thymoma – diagnosis; Thymoma – pathology; DNA neoplasms – analysis; Case report*

Introduction

Thymoma is an uncommon neoplasm derived from thymic epithelium. It is located in the anterosuperior mediastinum. Thymoma was found to be associated with different types of additional primary malignancies, of which colorectal adenocarcinoma and thyroid cancer appear to be most common[1,2]. The concurrence of thymoma and other malignancies could be the result of immune system disorders. On the other hand, *de novo* tumors following radiotherapy and chemotherapy administered for previously diagnosed neoplasms may be expected. One should always exclude genetic predisposition, history of carcinogen exposure, alcohol abuse, smoking habit, etc.

We describe a case of a woman with thymoma and two other primary neoplasms.

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Case Report

A 63-year-old woman was admitted to the hospital under the diagnosis of lung tumor. Three years before, the patient had undergone surgery for breast cancer. She was treated with chemotherapy. Two and a half years before this admission, the patient had a well circumscribed mass of unknown origin, detected on radiological investigation in the apical region of the left lung. Extensive clinical investigation followed and adenocarcinoma of the rectum was detected. She underwent surgical resection and was again treated with chemotherapy for colorectal adenocarcinoma. The mass in the lung was stable but seemed to have progressed during the past few months. It was decided to perform surgical resection of the lung mass; however, during preoperative examination another tumor mass was detected in the posterior mediastinum. Both mediastinal and lung tumors were resected, and metastatic lung carcinoma and mediastinal thymoma were diagnosed. One year after the last operation, the patient is well, without any evidence of the disease.

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Material and Methods

Tissue specimens from the breast and axilla were reexamined. Tissue specimens from the colon, lung and thymus were paraffin-embedded, formalin-fixed and microscopically examined using hematoxylin-eosin stain. Antibodies against estrogen and progesterone receptors (DAKO, Glostrup, Denmark) were used on immunohistochemical analysis. Mediastinal tissue was first decalcified and then paraffin embedded.

DNA ploidy pattern was analyzed by flow cytometry in the thymoma, lung tumor, breast carcinoma and colorectal adenocarcinoma. Nuclei were extracted from the most representative formalin-fixed and paraffin-embedded blocks using the method of Hedley *et al.*[3]. Briefly, three 50-μm sections were dewaxed in 4 mL of xylene at room temperature, rehydrated in a series of decreasing concentrations of ethanol, and washed in distilled water. Tissue fragments were incubated for 1 h in 0.5% pepsin (Sigma, Steinheim, Germany), pH 1.5 at 37 $^{\circ}$C in shaking water bath. Cells were filtered through a 42-μm filter. After washing and Rnase (Sigma, Steinheim, Germany) treatment (1 mg/mL in 37 $^{\circ}$C water bath for 30 minutes), the extracted nuclei were stained with propidium iodide (Sigma, Steinheim, Germany) in a final concentration of 50 μg/mL and incubated for 30 minutes at room temperature in the dark.

Cellular DNA content was analyzed on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, USA) by using excitation wavelength of 488 nm and 15 mW argon ion laser. For each DNA analysis, 20,000 nuclei were counted. DNA histograms were analyzed with a ModFitLT V3.0 (Verity Software House Inc., Topshame, ME and Becton Dickinson).

The status of the HER2 oncogene was examined in breast carcinoma and colorectal adenocarcinoma using chromogenic in situ hybridization technique (CISH, Invitrogen-Zymed Laboratories, San Francisco, USA). Paraffin-embedded sections were mounted on positively charged slides and cooked in a 60 $^{\circ}$C microwave oven for 3 hours. Tissue sections were deparaffinized and rehydrated. Boiling was performed at 98 $^{\circ}$C for 15 minutes in CISH Tissue Heat Pretreatment Solution in microwave oven, washed in distilled water and digested in Enzyme Pretreatment Reagent at room temperature for 10 minutes. The sections were again washed in distilled water, dehydrated in increasing concentrations of alcohol and air dried. HER2 probe (15 µL) was applied to the sections, overlapped and sealed. After denaturation at 95 $^{\circ}$C for 5 minutes, hybridization was performed overnight at 37 $^{\circ}$C. The next day coverslips were removed and the sections were rinsed in SSC buffer briefly at room temperature and for 5 minutes at 75 $^{\circ}$C. Signal detection was performed by immunodetection procedure, as described by the manufacturer (Initrogen-Zymed). Briefly, the slides were incubated with mouse anti-Dig antibody and HRP-anti mouse antibody and DAB chromogen at the end, hematoxylin counterstained and coverslipped.

HER2 gene signals were evaluated in tumor nuclei of counterstained specimens using 40x objective in 20 areas. Two hybridization signals *per* cell were described as not amplified (diploid). Cases with more than 10 signals were scored as amplified. High amplification was
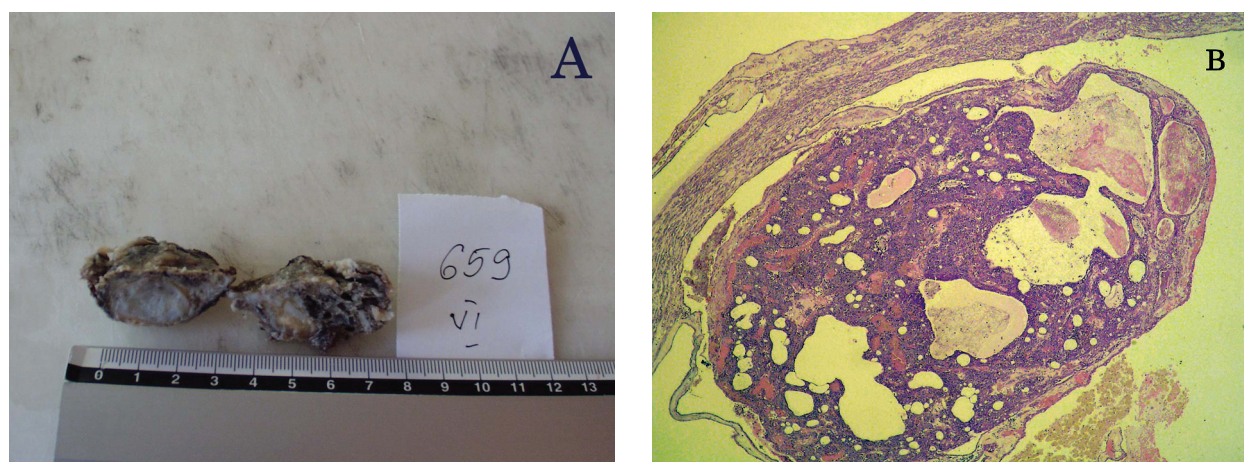

Fig. 1. (a) Gross features of thymoma found in a 63-year-old woman; (b) partly cystic tumor composed of uniform spindle and oval cells with bland nuclei. (H&Ex40)

described as more than 10 dots, large clusters or mixture of multiple dots and large clusters in at least 50% of cells in the chosen area. Low amplification was described as 5-10 dots or small clusters or mixture of multiple dots and small clusters in >50% of tumor cells.

Results

The patient underwent resection of the left upper lung lobe. The lung measured 17x10x4 cm. A solid, yellowish-gray tumor was detected in the periphery. It measured 2x1x1.5 cm. Histologically, it was composed of malignant glandular structures. The tumor was mostly necrotic. Tumor tissue was not found in 9 lymph nodes isolated from peribronchial and hilar tissue. In the same act, another tumor was resected from the mediastinum. The tumor measured 5.5x4.5x3 cm, and was quite solid and yellowish-brown. Grossly, it was composed of many cystic spaces (Fig. 1a). Histologically, cystic spaces were lined with epithelial cells that were also found within fibrovascular stroma. Epithelial cells were round with eosinophilic cytoplasm and pale nuclei having dispersed chromatin showing few mitoses (Fig. 1b). Epithelial cells were immunohistochemically positive for cytokeratin and EMA. Fibrovascular stroma between cystic spaces was abundant, with many calcifications, connective tissue, fat tissue, vascular spaces lined with endothelial cells which showed CD 34 and factor VIII positivity. Immunohistochemically, tumor cells were negative for vimentin, S-100 and CEA. The tumor was diagnosed as thymoma type A according to WHO classification.

An aneuploid pattern was found by flow cytometry in the lung tumor cells, with diploid population 88.13%, aneuploid 11.87% and total S phase 16.32%; DNA index 1.52 (Fig. 2a). The thymoma was diploid with G1 phase 95.28%, G2 phase 0.00% and S phase 4.72% (Fig. 2b).

We found no amplification of HER2 in lung tumor cells, while thymoma tissue could not be analyzed, probably because of DNA destruction during the process of decalcification.

The breast cancer measured 4 cm and was located on the edge of the mediolateral lower quadrant of the left breast. It was grayish-yellow and solid. Histologically, the tumor was composed of sheets and bands of atypical epithelial cells in desmoplastic stroma. Tumor cells showed polymorphism and hyperchromasia. There were up to 20 mitoses *per* 10 high power fields. Many tumor thrombi were found in vascular spaces outside the main tumor mass. Below the mammilla in breast parenchyma, another microscopic tumor mass was found, and was diagnosed as ductal invasive carcinoma. Nineteen lymph nodes were isolated from the axillary tissue and 12 of them had tumor metastases. We analyzed the expression of estrogen and progesterone receptors by immunohistochemistry. H score for estrogen was 190 and for progesterone 0. DNA ploidy analysis revealed aneuploid pattern: low percentage of aneuploid population of 8.01%, diploid population 91.88% and total S phase 3.82%; DNA index 1.71 (Fig. 2c). Low amplification of HER2 oncogene was found by CISH method (about 25% of tumor cells showed amplification).

Upon detection of colon carcinoma, the patient underwent second surgical resection; 42 cm of colon were resected. Two separate carcinomas were detected. One was located 3 cm from the nearest surgical resection margin. It was ulcerated and measured 2.5 cm in the largest diameter. The other one was close to the other resection margin. It was partially ulcerated and exophytic, measuring 2.8 cm in the largest diameter. The former tumor was composed of malignant glands infiltrating subserous fat tissue and was diagnosed as adenocarcinoma (Dukes B, Astler-Coller B2). Twelve lymph
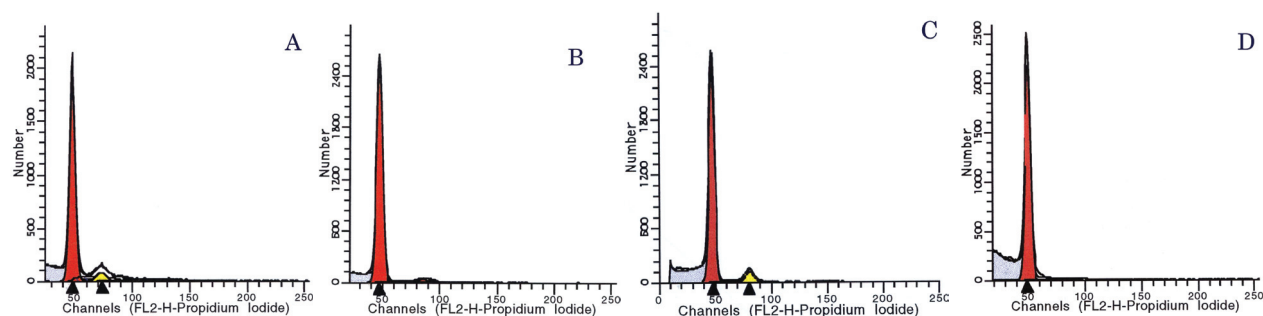

Fig. 2. DNA ploidy of multiple primary tumors including lung metastasis of colon carcinoma (A), thymoma (B), breast cancer (C) and colon carcinoma (D).

nodes were detected beneath the former tumor. They measured up to 0.5 cm and were free from tumor. However, malignant glands were detected in all lymph nodes beneath the latter tumor, which was classified as Dukes C, Astler-Coller C2. A normal diploid pattern was seen in the sample of colon carcinoma: G1 phase 96.11%, G2 phase 0.00%, S phase 3.89% (Fig. 2d). No amplification of HER2 gene was observed in colon carcinoma.

Discussion

One of the first reports on the association of thymoma with other primary malignancies came from the Mayo Clinic and Mayo Foundation, published in 1968[4]. In this paper, Souadjian *et al.* reported on 21% (31 of 146) of patients with thymoma to have also been diagnosed with a nonthymic malignant lesion[4]. According to a report from the John Hopkins Hospital, additional neoplasms were detected in 31% of thymoma patients[1]. Other large series have also confirmed higher incidence rates (8%-21%) of other primary neoplasms in association with thymomas[2]. In humans, additional malignant neoplasms described in association with thymoma were mostly colorectal adenocarcinoma and thyroid cancer[1].

The underlying mechanism of the increased risk is still being speculated. One of the possible causes proposed is radiotherapy after a thymoma has been diagnosed. Welsh *et al*.[1] could not confirm an increased risk of second malignancy after radiation therapy for thymoma. Another possible explanation of the increased risk is related to the role of the thymus as an organ of immune surveillance[2]. Some studies demonstrated histological differences in thymic tissue between cancer patients and normal controls[5]. The authors postulate that the development of thymoma implies a defect in thymic epithelium that hinders T-cell development, leading to immune defects and higher incidence of cancer. Some thymoma patients reportedly have peripheral T-cell lymphocytosis, which may reflect impairment of systemic immunoregulation accompanying thymic neoplasia[6]. Skinnider *et al*.[7] suggest that decreased NK cell function resulting from increased suppressor T-cell activity could be causally linked to the increased incidence of cancer in thymoma patients. Regarding the oncogenic tendency of patients with thymoma and other malignancies, no familial or hereditary syndrome connected with thymoma has been documented so far. Studies trying to connect any specific cytogenetic or molecular mechanism with thymoma are lacking. The clinical course of patients with thymoma varies widely despite its histologically benign appearance. In some studies, DNA ploidy was performed in a larger number of thymomas; however, no definitive ploidy pattern was established for different histologic types of thymoma. Pollack *et al.*[8] showed the higher recurrence rate to be consistently associated with aneuploidy and suggest that DNA index may be a useful parameter for identifying patients at a high risk of relapse. However, in our case a diploid pattern was identified yet the patient had two more primary tumors. All these tumors showed different DNA ploidy patterns in the same patient with invasive ductal carcinoma of the breast with aneuploidy and colon carcinoma again with a diploid pattern. On thorough English literature search using PubMed and Scopus databases, we found no similar data on DNA ploidy pattern in patients with thymoma associated with multiple primary tumors in the same patient.

On the basis of previous studies and our patient, it is concluded that the occurrence of extrathymic malignancies does not correlate with the biologic behavior of thymoma and shows no predilection for a specific thymoma subtype. Furthermore, many thymomas in patients with second tumors have been interpreted as benign. Awareness of this fact should urge clinicians to follow the patients with benign thymoma more regularly.

Although thymomas usually occur in the anterosuperior mediastinum, the one in our patient was located in the posterior mediastinum. Searching the literature we found no case of other thymoma at the same location and associated with multiple other malignancies.

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Sažetak

PLOIDNOST DNA U TIMOMU I PRIDRUŽENI VIŠESTRUKI PRIMARNI MALIGNITETI U ISTOG BOLESNIKA

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Timom je rijetka neoplazma podrijetlom iz epitela timusa. Smješten je u prednjem gornjem medijastinumu. Timom može biti udružen s različitim vrstama dodatnih primarnih maligniteta, no čini se kako su najčešći kolorektalni adenokarcinom i rak štitne žlijezde. Opisuje se 63-godišnja bolesnica s timomom tipa A i dvama drugim primarnim karcinomima dojke i kolona koji su bili prethodno dijagnosticirani. Bolesnica je operirana zbog raka kolona koji je metastazirao u pluća, te je pronađen još jedan primarni tumor u medijastinumu, koji je kasnije dijagnosticiran kao timom. Normalna diploidnost je utvrđena u uzorcima timoma i raka kolona, dok je rak dojke i metastatski tumor pluća pokazao aneuploidnost. Na osnovi ranijih studija i ovoga slučaja se zaključuje kako pojavnost maligniteta izvan timusa ne korelira s biološkim ponašanjem i ploidnošću DNA timoma.

Ključne riječi: *Novotvorine timusa – dijagnostika; Timom – dijagnostika; Timom – patologija; DNA novotvorine – analiza; Prikaz slučaja*