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CASE REPORT

Bcl-2 and MALT1 Genes are not Involved in the Oncogenesis of Uterine Tumors Resembling Ovarian Sex Cord Tumors

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Uterine tumors resembling ovarian sex cord tumors (UTROSCT) are rare entities. They were described by Clement and Scully in 1976 who classified them into groups I and II. Group I comprises typical endometrial stromal neoplasms with focal areas resembling ovarian sex cord elements and group II are predominantly or completely composed of ovarian sex cord-like elements. We report a case of UTROSCT type II with cytogenetic analysis. The tumor occurred in a 76-year-old woman who presented with vaginal bleeding. The tumor was lobulated, firm, yellow and histologically composed of sex cord-like elements. Tumor cells expressed vimentin, CD10, CD99 and alpha-actin. Cytogenetic analysis in a previously reported case detected translocation t(4;18)(q21.1;q21.3) in the majority of cells. Bcl-2 and MALT1 genes are located at or near the translocation breakpoints, and the aim of this study was to determine whether these genes were involved in chromosomal translocation or tumorigenesis. We did not find IgH translocation or the most common MALT translocations. Bcl-2 was also not involved in this oncogenesis. (Pathology Oncology Research Vol 13, No 2, 153–156)

Key words: uterus, ovarian sex cord tumors, Bcl-2, MALT1, translocation

Introduction

Uterine tumor resembling ovarian sex cord-like tumor (UTROSCT) is a rare tumor. These tumors were first described by Clement and Scully in 1976 who presented 14 cases of such tumors and classified them into groups I and II.[3] The groups are defined by the amount of sex cord-like elements. According to the available literature there have been only 55 cases reported so far and 19 were classified as group II UTROSCT.[9] Group I tumors (also known as endometrial stromal tumors showing focal sex cord-like differentiation – ESTSCLE) are endometrial stromal tumors (nodules or sarcomas) with less than 40% of sex cord-like elements. Group II tumors have more than 40% of sex cord-like elements with less endometrial component. They are usually surrounded by myometrium and sometimes lack any connection with the overlying endometrium. The nature of type II tumors is unclear, but since they comprise epithelial-like structures similar to those in type I tumors, some or all may be of endometrial stromal origin.[3]

Clinically, patients' age ranged from 24-86 years. Most of the women presented with abnormal vaginal bleeding or enlarged uteri. Macroscopically, these tumors resemble leiomyomas but lack their whorled pattern, are more lobulated and yellow. In two cases, a microcystic pattern has been described.[10,13] Their localization is myometrial, submucous and subserous, but two cases with cervical localization have also been reported.[12,16] The histologic features of ovarian sex cord-like elements are described as plexiform cords, trabeculae, nests or may form tubules with lumen. The stromal component in group I tumors occasionally exhibits mitotic activity (1-11 mitoses per ten high-power fields).

The biological behavior is variable. It has been noted that the group I tumors more often recur and metastasize. Their clinical behavior and prognosis depend on the stromal component (the number of mitoses or eventual vascular invasion). Group II tumors behave more benign without recurrence after surgery.

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Surgical excision is the treatment of choice in group II tumors and, if necessary, additional chemotherapy and irradiation are performed in group I tumors.

Wang et al[17] performed cytogenetic analysis on one UTROSCT. They detected chromosomal translocations t(4;18)(q21.1;q21.3) and t(X;6)(p22.3;q23.1) and suggested that the region on chromosome 18, which includes Bcl-2 and MALT1 genes, was involved in the new chimeric gene. Translocations t(11;18)(q21;q21) API2/MALT1 and t(14;18)(q32;q21) IgH/MALT1 are specifically associated with MALT lymphoma,[6] while translocation t(14;18)(q32;q21) IgH/Bcl-2 is most frequently found in follicular lymphoma.[17] In this case analysis we were trying to find out if there was any connection between these genes and the oncogenesis of UTROSCT.

Case report

The 76-year-old woman was admitted to the hospital due to vaginal bleeding. Her past medical history was almost unremarkable except for regulated hypertension, and gall bladder stones surgically resected 24 years ago. One month before admission to the hospital, complete curettage was performed because of vaginal bleeding. Fragments of inactive endometrial epithelial cells without stromal component were found. All laboratory tests with the exception of CA 125 (86.0 kIU/L; normal values 0-35 kIU/L) were within normal range. The uterus was firm. On ultrasound a hyperdense, solid mass was found in the posterior wall of the uterus. It measured 70x50 mm. Surgical excision was recommended and total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed. The patient is currently alive and free of disease 48 months after the surgery.

Materials and Methods

Immunohistochemistry

Formalin-fixed, paraffin-embedded sections (2-4-µm-thick) were used for histopathological examination and stained with hematoxylin and eosin, Gomori or periodic acid-Schiff (PAS). Immunohistochemical stainings were performed and revealed with the LSAB-HRP (DAKO, Glostrup, Denmark) detection system for epithelial membrane antigen (EMA, DAKO, clone E29, 1:100), desmin (DAKO, clone D33, 1:200), alpha-actin (DAKO, clone 1A4, ready-to-use), cytokeratin (DAKO, clone MNF 116, 1:100), Melan-A (DAKO, clone A103, 1:10), cytokeratin AE1-AE3 (DAKO, code M3515, 1:50), vimentin (DAKO, clone V 3B4, 1:100), CD10 (Novocastra, clone 56C6, 1:20), S100 (DAKO, code 311, 1:1000), CD34 (DAKO, code QBEnd10, 1:25), CD99 (DAKO, myc2 gene, clone 12E7, ready-to-use), Bcl-2 (DAKO, clone 124, 1:40), placental alkaline phosphatase (PLAP, DAKO, clone 8B6, 1:25), CA 125 (DAKO, clone M11, 1:20), inhibin (DAKO, clone R1, 1:25) and CD31 (DAKO, clone JC/70A, 1:40).

Fluorescent in situ hybridization (FISH)

Formalin-fixed, paraffin-embedded sections (4-µm-thick) were deparaffinized using Histoclear (National Diagnostics, England), rehydrated in an alcohol series (100%, 85% and 70%, 2 min each), washed with distilled water, cooked in antigen retrieval solution (Target Retrieval Solution, DAKO) (15 min in microwave oven), treated with pepsin for 20 min at 37ºC, dehydrated in a graded series of alcohol (70%, 85% and 100%, 2 min each), and allowed to air dry in the dark. Dual fusion t(14;18)(q32;q21) IgH/Bcl-2, t(14;18)(q32;q21) IgH/MALT1, t(11;18)(q21;q21) API2/MALT1 and IgH break-apart FISH probes (Vysis Inc., USA) were prepared according to the manufacturer's instructions. An aliquot (2 µl) was applied to the area of interest on the sample and covered with a coverslip. Slides were placed in a Hybridizer (DAKO) and denatured at 82ºC for 5min, followed by overnight hybridization at 45ºC. The next day, slides were washed in preheated 0.003% Tween/0.4xSSC at 72ºC for 2 min, then in preheated 0.001% Tween/2xSSC at 72ºC for 1 min and in 2xSSC at room temperature for 2 min. Slides were mounted using Vectashield mounting medium (Vector Laboratories Inc., USA) with 10^{-5} g/l DAPI (Serva, Germany) and viewed using a fluorescence microscope.

Results

Pathological findings

The uterus measured 14x10x7 cm. A fairly well-circumscribed, yellow, firm tumor, measuring 7.5 cm was found in the myometrium (in the posterior wall of the uterus). Histologically, the lesion was well circumscribed but unencapsulated with pushing but not infiltrating borders towards the surrounding myometrium. The tumor did not involve the overlying inactive endometrium. It was mostly (>70%) composed of tubular formations and anastomosing trabeculae with scant stromal component among them. The tumor cells were small to medium sized with round to oval nuclei which exhibited slight hyperchromasia and pleomorphism. Their cytoplasm was scant and eosinophilic. Mitotic activity, lymphatic or vascular involvement was not found.

The cervix, the uninvolved uterus, the ovaries and fallopian tubes were histologically unremarkable. Immunohistochemically, the tumor cells were positive for vimentin (*Fig. 1*), CD10 and CD99 (*Fig 2.*), focally positive for alpha-actin and negative for EMA, desmin, cytokeratins, Melan-A, S100, CD34, Bcl-2, PLAP, inhibin, CA 125 and CD31.

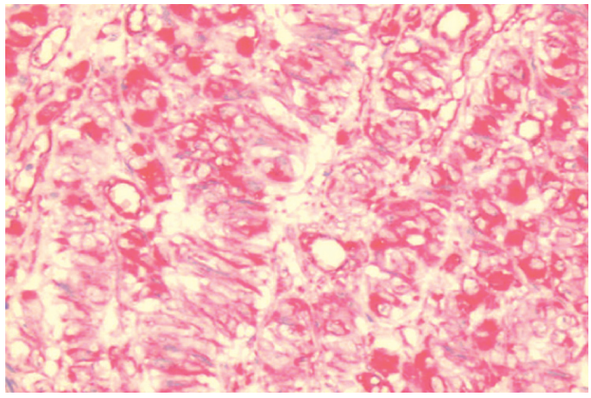

Figure 1. *Tumor cells are immunohistochemically positive for vimentin*

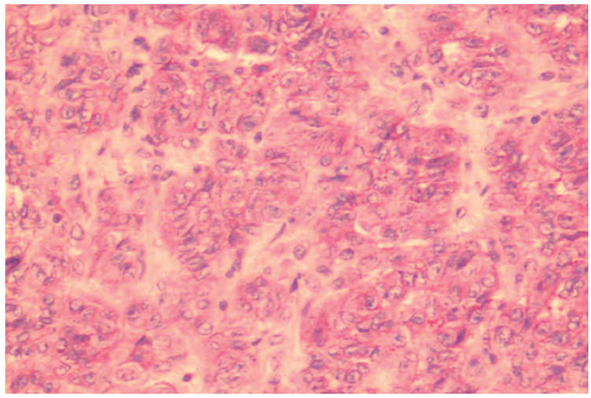

Figure 2. *Tumor cells are immunohistochemically positive for CD99*

DNA content analysis showed diploid pattern with 85.45% of tumor cells being in G0/G1 phase. The fraction of S phase was 9.43%.

Analysis of FISH signals showed lack of IgH gene translocation and lack of IgH/Bcl-2, IgH/MALT1 and API2/MALT1 translocations.

Discussion

According to Wang et al[17] who reported strong immunoreactivity for Bcl-2, we immunostained our sample for Bcl-2 and searched for IgH/Bcl-2 translocation, with negative results. We also wanted to check if there could be any connection with the oncogenesis of lymphomas and abnormality of the MALT1 gene in relation to the pathway of development of the second group UTROSCT. Wang et al detected translocation which included a region on chromosome 18. A few well-known tumor-related genes are located in this region. 18q21 encompasses Bcl-2, MALT1, FVT1, SCCA1, SCCA2 and DCC. We investigated the most common MALT1 gene translocations. We did not find either IgH translocation or the most common MALT translocations. We found no evidence that the development of the second group of UTROSCT involves the MALT1 gene. Likewise, there was no evidence that Bcl-2 is involved in this oncogenesis at all.

Ever since defining the two groups of these tumors there has been controversy regarding the true nature of these formations. The development of immunohistochemistry over the decades has in many ways enlightened some of the doubts. Sex cord tissue resembles endometrial stromal cells which are CD10-positive, and myofibroblastic cells, which is proved by a varying frequency of alpha-actin positivity. In all recent studies and case reports, including ours, there has been variable positivity for vimentin, cytokeratin, smooth muscle actin, desmin, Melan-A, inhibin and CD10.[1,6,14,16,17]

The most important discoveries were published in the studies of Krishnamurty et al[14] and Baker et al.[1] These studies were focused on immunohistochemical findings in UTROSCT group II with an emphasis on inhibin and CD99. CD99 is a marker for granulosa cell tumors, Sertoli cell tumors and others. Busam et al[2] discovered Melan-A positivity in steroid-producing cells and tumors composed of such cells located in the adrenal cortex, testis and ovary. In Krishnamurty's group of seven tumors, 3 were inhibin-positive, 4 Melan-A-positive and all of them were positive for CD99.[14] In Baker's study[1] all five cases were positive for inhibin and CD99. Sixteen tumors in other studies[5,6,14,16,17] were CD99-positive, while only one of them was diffusely, weakly positive.

All these findings support the suggestion of Mazur et al[15] that uterine tumors with different histology actually share a common histogenetic origin representing the capacity of uterine mesenchymal blastema of muellerian origin to differentiate; in the case of UTROSCT into epithelial, myoid and sex cord-stromal component with varying frequency.

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