An unusual case of late recurrent adult granulosa cell tumor and mature teratoma arising within the same ovary, confirmed by NGS analysis

Adam Šafanda, Nikola Hájková, Jan Galko, Michaela Kendall Bártů, Pavel Dundr, Kristýna Němejcová

Department of Pathology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic

SUMMARY

Adult granulosa cell tumor is a predominant malignant tumor among ovarian sex cord-stromal tumors, representing approximately 3-5% of all ovarian malignancies and being known for its risk of recurrence with high mortality rate. We present a unique case of a 71-year-old woman with, to our knowledge, the first documented instance of a recurrent AGCT arising concurrently with a mature ovarian teratoma, confirmed through both immunohistochemistry and molecular biological analysis. The tumor in both the primary and recurrent lesion harbored a missense *FOXL2* mutation typical for adult granulosa cell tumor. *TP53*, *TSC2* and *RB1* mutations were present only in the recurrent tumor, indicating secondary mutations acquired during progression.

Keywords: sex cord-stromal tumors – adult granulosa cell tumor of the ovary – teratoma – NGS – ovary

Neobvyklý a molekulárně-biologicky verifikovaný případ recidivujícího adultního nádoru z buněk granulózy vznikající společně se zralým teratomem ve stejném ovariu

SOUHRN

Adultní nádor z buněk granulózy je nejčastějším maligním gonadostromálním nádorem ovaria, představuje přibližně 3–5 % všech malignit ovaria a je známý svým rizikem recidiv spojených s vysokou mortalitou. Představujeme zajímavou kazuistiku 71leté ženy s doposud prvním zdokumentovaným případem recidivujícího adultního nádoru z buněk granulózy vznikajícího společně se zralým teratomem ve stejném ovariu, potvrzeným jak imunohistochemií, tak molekulárně-biologickou analýzou. Nádor vykazoval v primární i recidivující lézi missense mutace v genu *FOXL2*, která je typická pro adultní nádor z buněk granulózy. Mutace v genu *TP53*, *TSC2* a *RB1* byly přítomny pouze v recidivujícím nádoru, což naznačuje sekundární mutace získané během progrese.

Klíčová slova: sex cord-stromální nádory – adultní nádor z buněk granulózy – teratom – NGS – ovarium

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Ovarian sex cord-stromal tumors comprise a heterogeneous group of neoplasms characterized by variable biological behavior, classified into three categories according to the cellular origin; including pure stromal tumors, pure sex cord tumors, and mixed sex cord-stromal tumors (1). Adult granulosa cell tumor (AGCT) is the most prevalent malignant subtype within the sex cord-stromal tumor group, accounting for approximately 3% to 5% of all ovarian malignancies. A subset of ovarian sex cord-stromal tumors exhibits uncertain biological behavior, with AGCT in particular prone to late recurrences which significantly reduce the survival rate (2-3). Recent advancements in molecular biology have facilitated the resolution of challenging cases where morphological and immunohistochemical features overlap, for example in case of missense *FOXL2* (c.402C>G, p.Cys134Trp) mutation which is mostly found in AGCT and a subset of Sertoli Leydig cell tumors (SLCT), as described in our recent publication (4-6). Mature teratomas are a type of germ cell tumor and represent one of the most common ovarian neoplasms. They are characterised by the presence of differentiated tissues derived from all three germ layers: ectoderm, mesoderm, and endoderm (1,7). Mature teratomas are benign tumors, in contrast to immature teratomas, which are malignant and are graded based on the amount of immature neuroectodermal tissue (1). However, malignant transformation of mature teratoma occurs in approximately 2% of cases with squamous cell carcinoma being the most common type of somatic malignancy arising in a mature teratoma (8). Various other somatic malignancies have been documented arising within teratomas, including rare cases of sex cord-stromal tumors, exclusively AGCT (7,9). Nevertheless, most of the existing literature describes AGCT and mature teratoma as independent synchronous neoplasms, in contrast to secondary somatic malignancies arising within a pre-existing mature teratoma (7). We present a unique case of a 71-year-old woman with, to our knowledge, the first documented instance of a recurrent AGCT arising concurrently with a mature ovarian teratoma, confirmed through both immunohistochemistry and molecular biological analysis.

✉ ***Correspondence address:***
Doc. MUDr. Kristýna Němejcová, Ph.D
Department of Pathology
First Faculty of Medicine, Charles University and General University Hospital in Prague
Studničkova 2, 12800 Prague 2, Czech Republic
tel.: +420224968632
e-mail: kristyna.nemejcova@vfn.cz

CLINICAL HISTORY

The patient was a 71-year-old female with diffuse large B-cell lymphoma (DLBCL) in personal history, who underwent bilateral adnexectomy and hysterectomy in 2007 with an incidental

finding of a mature cystic teratoma of the left ovary. In 2023 imaging revealed a sigmoid colon mass and multiple liver lesions suspicious for metastases, which were treated by resection of the sigmoid colon and a biopsy of the liver lesions, followed by subsequent chemotherapy. As of November 2024, the patient remains alive with active disease and is currently undergoing chemotherapy to manage the liver metastases.

MATERIALS AND METHODS

Immunohistochemical analysis

Immunohistochemical (IHC) analysis was performed using 4-µm-thick sections of formalin-fixed and paraffin-embedded (FFPE) tissue. The list of antibodies used, their clones, manufacturers, dilution, and staining instruments is summarized in Table 1.

Molecular analysis

Genomic DNA was isolated from FFPE tissue from the recurrent AGCT (estimated tumor purity was 95 % of tumor cells) and from the AGCT associated with a mature teratoma of the ovary (80 % of tumor cells) using the Quick-DNA/RNA FFPE Miniprep Kit (Zymo Research), according to the manufacturer's protocol.

Sequence capture NGS analysis of the DNA was performed using the KAPA HyperPlus kit according to KAPA HyperCap Workflow v3.0 (Roche) and a panel of hybridization probes against multiple targets of cancer relevant genes (360 genes or gene parts; 951 kbp of coding regions; Roche). Unique Molecular Identifiers (UMIs) were incorporated during library preparation in the primary tumor (from 2007) to remove sequencing errors, unmasking low-frequency variants. The prepared sample libraries were pair-end sequenced by the NextSeq 500 instrument (Illumina) using NextSeq 500/550 High Output Kit v2.5 (Illumina). The biostatistical evaluation was performed using CLC Genomics Workbench software (CLC GW; Qiagen, Venlo, The Netherlands) and variants with mutation allele frequency (MAF) of at least 4 % were identified. Interpretation of the DNA variants was performed as described in a previous study (10). All variants were manually reviewed using the IGV software to eliminate potential sequencing artifacts or PCR duplicates and were analyzed and compared between both tumors. A variant with ≥ 1% MAF detected in the primary tumor was considered present if it was also detected in the recurrent tumor.

RESULTS

Morphological and immunohistochemical findings

The sigmoid colon mass measured 80x70x60 mm, presenting as a homogenous yellow mass with focal areas of necrosis, and was situated within the muscularis externa and subserosal layers of the colon. Histologically, the tumor exhibited features of AGCT with predominantly diffuse growth pattern, with areas of trabecular and alveolar structures and multiple necrotic areas. The tumor cells displayed eosinophilic cytoplasm and relatively uniform, larger vesicular nuclei with prominent nucleoli. In certain regions the tumor showed marked nuclear atypia, including the presence of monstrous nuclei and frequent mitotic figures. During the diagnostic evaluation of the sigmoid colon tumor, the left ovarian tumor diagnosed in 2007 as mature teratoma was re-examined, and the second reading showed focal areas of a tumor with AGCT features growing in a close proximity to the mature teratoma, which was made up of structures of a dermoid cyst, thyroid gland tissue, and mucinous epithelium. The biopsy of one of the liver lesions showed a tumor of the same morphology of AGCT as the mass in the sigmoid colon. Representative examples of the microscopic features (H&E staining) of the primary and recurrent tumors, along with the results of SF1 and p53 immunohistochemistry of the recurrent tumor are presented in Figure 1. The results of the immunohistochemical examination are provided in Table 1.

Table 1. List of the immunohistochemical antibodies used, including their clones, manufacturers, dilution, staining instruments, and a summary of the results in the primary and recurrent adult granulosa cell tumor.

Antibody	Clone	Dilution	Producer	Platform	Detection	Results (primary tumor)	Results (recurrence)
SF1	EPR 19744	1:400	Abcam, Cambridge, UK	Dako Omnis, (Agilent, Santa Clara, CA, USA	EnVision FLEX (Dako)	Diffuse and strong expression	Diffuse and strong expression
Calretinin	DAKCalret	1:00	Dako, Glostrup, Denmark	Dako Omnis, (Agilent, Santa Clara, CA, USA	EnVision FLEX (Dako)	Diffuse and strong expression	Moderate expression in 5% of cells
Inhibin A	R1	RTU	Dako, Glostrup, Denmark	Dako Omnis, (Agilent, Santa Clara, CA, USA	EnVision FLEX (Dako)	Diffuse and strong expression	Moderate expression in 5-10% of cells
FOXL2	Polyclonal Ra	1:200	Novus Bio, USA	Ventana BenchMark ULTRA (Roche, Basel, Switzerland)	OptiView	Weak to moderate extensive (80%) expression	Weak to moderate expression in 60% of cells
CKAE 1/3	AE1/AE3	1:200	Dako, Glostrup, Denmark	Dako Omnis, (Agilent, Santa Clara, CA, USA	EnVision FLEX (Dako)	Granular positivity in 50% of cells	Moderate expression in 30-40% of cells
p53	DO-7	1:400	Dako, Glostrup, Denmark	Ventana BenchMark ULTRA (Roche, Basel, Switzerland)	UltraView	Wild type	Aberrant
ER	SP1	1:200	Zytomed Systems GmbH, Berlin, Germany	Ventana BenchMark ULTRA (Roche, Basel, Switzerland)	OptiView	Weak expression in 10-20% of cells	Weak expression in 10-20% of cells

MAF: mutation allele frequency; AGCT: adult granulosa cell tumor

Molecular findings

A total of five pathogenic or likely pathogenic mutations in *FOXL2* (p.Cys134Trp), *RB1* (p.Cys712Ter), *TSC2* (c.2546-1G>C splice variant) and two mutations in *TP53* gene (p.Arg273Cys and p.Leu257Arg) were detected in the recurrent sex cord-stromal tumor. Only the *FOXL2* mutation and one *TP53* mutation (p.Leu257Arg) were also detected in the primary tumor with a low mutation allele frequency (MAF; 13% and 1%, respectively). Detailed list of the pathogenic or likely pathogenic variants is provided in Table 2.

Table 2. Detailed pathogenic or likely pathogenic variants detected in both tumors.

			Primary AGCT	Recurrent AGCT
gene	genomic level	protein level	MAF (%)	MAF (%)
FOXL2	NM_023067.4:c.402C>G	p.Cys134Trp	13	46
RB1	NM_000321.2:c.2136C>A	p.Cys712Ter	not detected	87
TSC2	NM_000548.5:c.2546-1G>C	splice site	not detected	28
TP53	NM_000546.5:c.817C>T	p.Arg273Cys	1	45
TP53	NM_000546.5:c.770T>G	p.Leu257Arg	not detected	45
			estimated tumor purity 80%	estimated tumor purity 95%

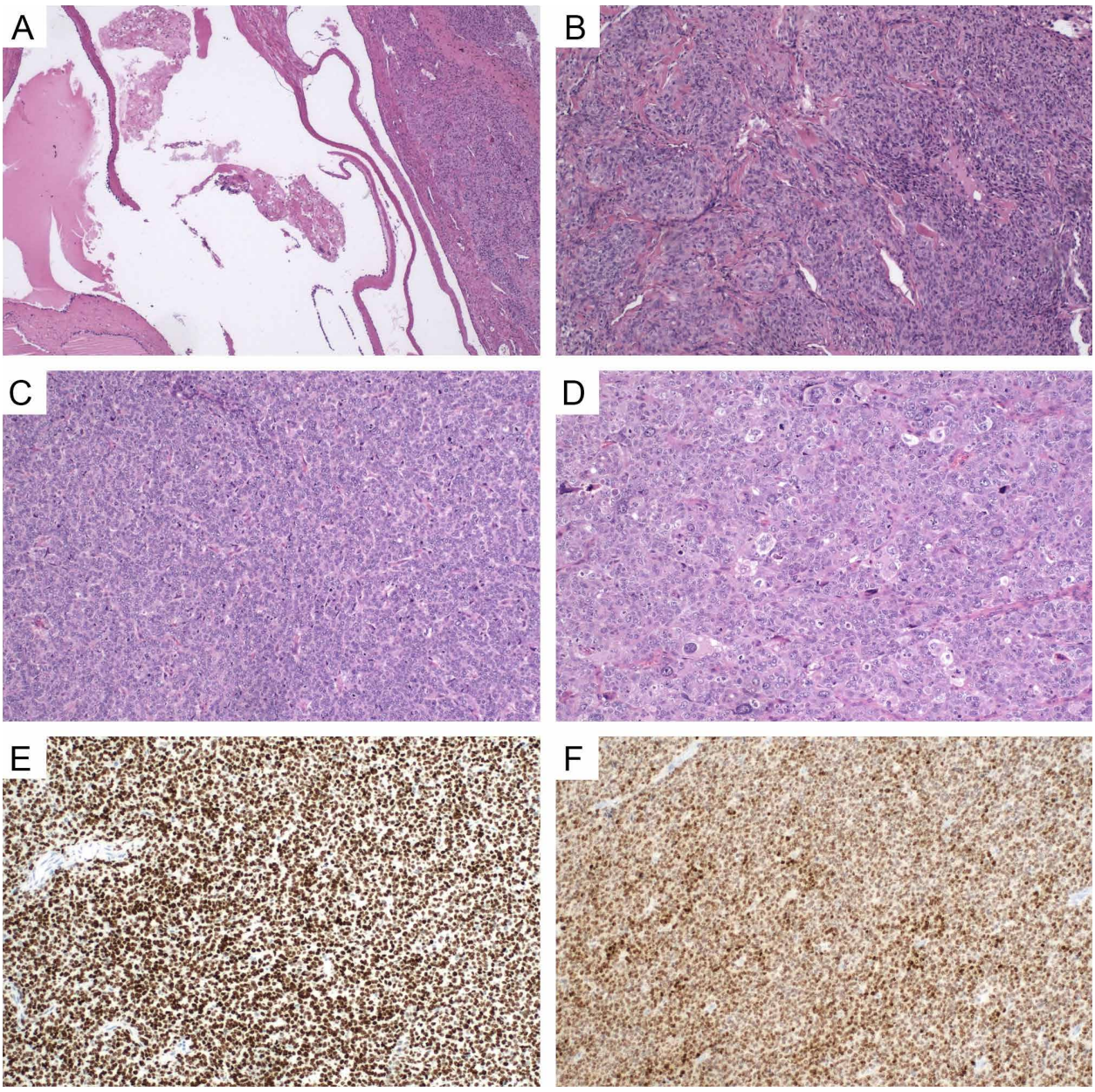

Fig. 1. The representative morphological and immunohistochemical features of the tumors. **A:** Overview of the primary tumor (right) in close proximity to the structures of a mature cystic teratoma (left), 40x magnification (H&E staining). **B:** Histomorphology of the primary tumor, 100x magnification (H&E staining). **C:** Histomorphology of the recurrent tumor, 100x magnification (H&E staining). **D:** Histomorphology of the recurrent tumor (monstrous nuclei), 100x magnification (H&E staining). **E:** Aberrant p53 immunohistochemistry in the recurrent tumor, 100x magnification. **F:** Strong nuclear positivity of SF1 in the recurrent tumor, 100x magnification.

DISCUSSION

Sex cord-stromal tumors and mature teratoma developing within the same ovary simultaneously are exceedingly rare, with just over ten cases reported in the literature to date and the oldest dating back to 1919 (11). The difficulty lies in determining whether the tumor originates within the mature teratoma itself or develops independently as a synchronous neoplasm within the same ovary. This question can be comprehensively resolved through thorough sampling and the application of molecular pathology methods comparing the genetic landscape of both tumors. In most studies, the diagnosis was made solely based on morphological assessment using standard hematoxylin and eosin-stained slides, and only a few recent studies have incorporated immunohistochemistry into the diagnostic process (12-13). All previously documented cases have been diagnosed as granulosa cell tumors and no other type of sex cord-stromal tumor has been reported in this setting. We additionally provide a DNA NGS analysis to examine the molecular landscape of the primary and recurrent tumor. In our case, both the primary and recurrent tumor harbored the same missense *FOXL2* mutation. This mutation serves as a diagnostic feature of AGCT as it is present in a majority of cases (4). However, this mutation can also be observed in a subset of SLCT and, rarely, in other sex cord-stromal tumors, highlighting the challenging differential diagnosis due to significant histomorphological overlap with AGCT (5-6,14-15). Additionally, a *TP53* gene mutation was identified in the recurrent tumor, consistent with aberrant immunohistochemical p53 expression. Concurrent mutations of *FOXL2* and *TP53* have also been reported in other sex cord-stromal tumors, including sex cord-stromal tumor not otherwise specified (NOS) or gynandroblastoma (14-15). Regarding the molecular background of AGCT, Michálková et al. provided a complex analysis of 227 cases comparing primary and recurrent tumors and found *RB1* mutation in one recurrent case in addition to the *TP53*, *FOXL2,* and *TERT* mutations. The *TP53* mutation was detected in 3.5% of cases. However, in their cohort they did not detect any *TSC2* mutations (16). The low MAF of the detected variants in the primary tumor suggests that these mutations are confined to a smaller subpopulation of cells within the primary tumor. In contrast, the additional mutations identified exclusively in the recurrent AGCT (*TSC2*, *RB1,* and second *TP53* mutation) may represent secondary genetic events acquired during tumor progression, potentially explaining the increased aggressiveness observed in the recurrent tumor. In conclusion, we present the first molecularly characterized case of simultaneous ipsilateral ovarian AGCT and mature cystic teratoma with a late recurrence of the AGCT component. The tumor in both the primary and recurrent lesion harbored a missense *FOXL2* and *TP53* mutation typical for AGCT. *TSC2* and *RB1* mutations were present only in the recurrent tumor, indicating secondary mutations acquired during progression.

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STATEMENTS AND DECLARATIONS

Competing Interests: The authors declare no competing financial and/or non-financial interests relevant to the content of this article.

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