Case report

Analysis of mutations of *PARP1*, *RNF213*, *PAX8*, *KMT2C*, *MTRR* in malignant mesothelioma of testicular tunica vaginalis testis

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Molecular next gene sequencing was used to evaluate mutations in 409 common mutated cancer-related genes in malignant mesothelioma of tunica vaginalis testis (MMTVT) of 81-year-old man. Multifocal papillary-solid areas contained necrosis among highly cellular fields with multiple mitoses. It was positive for WT1, CKAE1/AE3, calretinin, CK7 with negativity for CK5, PSA, TTF-1. Following mutations were revealed in *PARP1* (NM_001618: c.2285T<C p.V762A), *RNF213* (NM_001256071: c.3121G<A, p.A1041T), *PAX8* (NM_013992: c.404A>G, p.K135R), *MTRR* (NM_024010: c.147A>G, p.I49M) and two sorts of mutations in structure of *KMT2C* gene (NM_170606: c.2447_2448insA (c.2447dupA), p.Y816fs and NM_170606: c.1042G>A, p.D348N) for the first time in MMTVT.

Key words: *PARP1*, *RNF213*, *PAX8*, *KMT2C*, *MTRR*, *KMT2C*, mesothelioma, testicular tunica vaginalis.

Introduction

We decided to present the case of malignant mesothelioma of tunica vaginalis testis (MMTVT) due to its rarity. Malignant mesothelioma of tunica vaginalis testis constitute less than 0,5% of the total of mesotheliomas of the human body [1, 2, 3]. Our case belongs to the category of mesotheliomas arising in coelomic cavities and such tumors of that counterpart of human body are truly rare with variety of histological patterns in the non-uniform group of neoplasms of that type in this anatomical setting [1, 2, 3]. Persistent hydrocele, herniorrhaphy, trauma, relapsing epididymitis are considered to be predisposing factors in the development of MMVT [4]. However, coexistence of these conditions could be more coincidence that real predisposition due to rarity of this tumor type in testicular region. Nevertheless, there is no doubt that the MMVTs are associated with exposure to asbestos (like in case pleural mesotheliomas) [5, 6]. The prognosis is dismal as – according to Recabal *et al.* – the tumor metastasize to lungs and groin with involvement of retroperitoneal and to lesser extend pelvic lymph nodes with deaths of five individuals from total studied group of 15 patients during 3.5-years-long follow-up [7]. There is little

known about molecular biology of this tumor at the site of tunica vaginalis testis. However, with appliance of whole-genome sequencing method, Zhang *et al.* have recently reported on mutations in mesothelioma-associated genes such as KIF25, AHNAK, and PRDM2 and stated that MMVT harbored C-to-T and T-to-C mutations as well as it was characterized by amplification of copy number in its chromosomes 1 and 12 [8]. Our current report presents a case of malignant mesothelioma of tunica vaginalis testis (MMTVT) with detected for the first time mutations of *PARP1*, *RNF213*, *PAX8*, *KMT2C*, *MTRR*, in such a tumor in this location.

Material and methods

The postoperative specimen contained testicle with epididymis enveloped in testicular tunicas and spermatic duct of 81-year-old man. The postoperative specimen contained: the testis of dimensions: 6 × 3 × 2.5 cm without macroscopically visible focal lesions and 6 cm-long epididymis, mean up to 0.5 cm. The space was grossly enlarged between thickened visceral and parietal laminas of testicular tunica vaginalis with evident hydrocele. There were also numerous exophytic papillary excrescences with the largest diameter up to 3 cm that were mostly located in upper pole of the testicle on the visceral lamina of the tunica vaginalis. The papillary excrescences coalesced with one another to extend to greatest dimension to 6 cm in length, but this value was not diameter of the neoplasm as there was no one solid spherical tumor, but instead there was a multitude of papillary foci that studded both visceral and parietal lamina of tunica vaginalis testis. The neoplastic excrescences did not macroscopically show any deep invasion with mainly smooth interface with surrounding tissues. Representatives samples were taken from areas of tumor masses of testicular tunica vaginalis and underwent immunohistochemical staining with WT-1 (mouse monoclonal anti-human antibody, WT49, PA0562, dilution: 1:100), CK7 (NCL-CK7 OVTL dilution: 1:200), Calretinin (mouse monoclonal anti-human antibody PA0346, Clone CAL6, Novocastra Labs, Dilution: 1:40), CKAE1/AE3 (Monoclonal Mouse Anti-Human Cytokeratin, Clone: AE1/AE3 Isotype: IgG1, κ. M3515, dilution:1:50), CK5 (mouse monoclonal anti-human antibody, NCL-CK5, Novocastra dilution: 1:100), PSA (mouse anti-human, monoclonal antibody, PA0431, Clone 35H9 Novocastra ready-to-use dilution to Bond system), TTF-1 (NCL-L-TTF-1, Clone SPT24 human thyroid transcription factor-1 (TTF-1), 1:50 dilution). Simultaneously gene profiling was performed. Status of hot spots found in 409 tumor genes were studied by means of next generation sequencing (NGS) technology (IonTorrent-Thermo Fisher Scientific, USA) using Ion AmpliSeq™ Comprehensive Cancer Panel. Detected mutations were annotated using COSMIC (https://cancer.sanger.ac.uk/cosmic), dbSNP database (https://www.ncbi.nlm.nih.gov/snp/) and Varsome database (https://varsome.com/).

Results

81-year-old man underwent total removal of testis, epididymis, testicular tunicas and part of spermatic cord due to clinically overt testicular hydrocele and later revealed papillary excrescences of tumor masses of vaginal tunica of the testis. The tumor was mainly composed of multifocal exophytic papillary-solid texture with necrosis fields among tightly packed areas of high cellularity with multiple mitoses, thus constituting a neoplasm of high grade malignancy. Actually, necrosis occupied 15% of tumor texture. Mitotic rate was 25 mitoses per 10 HPF on average. The cellular nuclei were predominantly oval, the chromatin was sparse in nuclei thus it was easy to notice that nuclei contained visible but not substantially enlarged nucleoli. The cytoplasm of neoplastic cells was rather eosinophilic with apparent shift toward amphophilic appearance in the highly cellular solid areas with high mitotic rate. The cells were cuboidal, oval and some of them a bit elongated. The cells were tightly packed in anastomosing rolls, rows and trabecules that streamed into more uniform solid areas without any discernible peculiar pattern, while on the periphery the cells were arranged in papillary-tubular glandular-like architecture. It occupied visceral lamina and to lesser extend parietal lamina of tunica vaginalis of testis with local invasion of rete testis without apparent extension beyond parietal lamina and without involvement of spermatic duct. Thus the tumor border and the interface of the tumor with surrounding tissue was rather smooth than infiltrative except from involvement of rete testis. The tumor was strongly positive for WT-1 (nuclear reaction) CKAE1/AE3 and calretinin (Fig. 1). It was also evidently positive for CK7 with negativity for CK5, PSA, TTF-1 as well as negative mucicarmine stain (Fig. 1). The tumor was diagnosed as malignant mesothelioma (epithelioid type) of the testicular vaginal tunica (pT2: 8th edition of pTNM staging) [9]. The use of next gene sequencing (NGS) enabled detection of six mutations in five genes (Table I). Namely, NGS revealed mutations in *PARP1* (NM_001618: c.2285T<C p.V762A), *RNF213* (NM_001256071: c.3121G<A, p.A1041T), *PAX8* (NM_013992: c.404A>G, p.K135R), *MTRR* (NM_024010: c.147A>G, p.I49M) and two sorts of mutations in structure of *KMT2C* gene (NM_170606: c.2447_2448insA (c.2447dupA), p.Y816fs and NM_170606: c.1042G>A, p.D348N) for the first time in this rare kind of tumor of genitourinary tract.

In the analysis four mutations were classified as benign polymorphism (PARP1 p.V762A, RNF213 p.A1041T and MTRR pI49M, KMT2C p.Y816fs) and two as pathogenic mutations (PAX8 p.K135R and KMT2C p.D348N) (Fig. 2).

Discussion

In our opinion our tumor fits best to category of diffuse malignant mesothelioma with prominent papillary features in differential diagnosis of spectrum of borderline tumors between classic well differentiated papillary mesothelioma and diffuse malignant mesothelioma [10]. In regard to detected molecular gene abnormalities in mesothelioma, one should be aware of the fact that detected polymorphism (p.V762A) in the PARP1 encoding protein which is involved in the repair of DNA damage and cell proliferation and death, in a meta-analysis it was associated with an increased risk of cancer in Asian populations, but with a reduced risk for Caucasian populations [11]. Another meta-analysis also confirmed an increased risk of neoplastic incidence for gastric, cervical, and lung cancers as well as for gliomas in carriers of p.V762A in Asians [12]. The study conducted on the Polish population indicates this polymorphism as a risk factor for the development of cervical cancer [13]. The above data imply that the significance of polymorphism might be associated with the presence of other genetic factors, including hereditary polymorphisms and somatic changes in some cancers like malignant pleural mesotheliomas (MPM) [14]. p.A1041T polymorphism in the *RNF213* (RNF213 possesses both ATPase activity and E3 ubiquitin-protein ligase activity) was detected in the present study. This polymorphism was previously described in the moyamoya population and control population [15]. The third of polymorphisms was detected in the *MTRR* gene, which codes for methionine synthase reductase, that is involved in the synthesis of methionine [16]. The polymorphism detected in the analyzed case p. I49M was previously described in the population with bone metastases of lung cancer [17]. Interestingly, deprivation of methionine is a potential strategy of oncological treatment analogously to arginine deprivation proposed for therapy of mesothelioma [18, 19, 20]. According to the bioinformatic analysis, the detected mutation in *PAX8* is pathogenic. PAX8 is a transcription factor associated with embryogenic development, particularly in modeling of reproductive organs [21]. It is also used as a useful diagnostic marker of the differentiation of ovarian tumors [21]. On the other hand, this mutation (COSM6466208) has been flagged as a SNP and excluded from COSMIC database. It should be added that only focal and/or weak staining for PAX8 was described in pleural malignant mesothelioma [22]. Methylation, demethylation and acetylation of histone proteins have implications in cancerogenesis. Histone modifications are responsible for regulating the availability of transcription factors and other functional proteins to chromatin, thereby regulating transcription, translation, replication, DNA repair and recombination of DNA [23]. KMT2C (Lysine N-methyltransferase 2C 2-lysine methyltransferase)/MLL3 (myeloid/lymphoid or mixed myeloid/lymphoid or mixed-lineage leukemia) is regarded a significant tumor suppressor, due to its mutations that lead to the reading frame change were very often detected in colorectal cancer [24]. Recently, Wang *et al.* detected cancer mutational hotspot in MLL3 within the region that encodes its plant homeodomain (PHD) repeats to evidence involvement of this domain in interaction with the histone H2A deubiquitinase as well as tumor suppressor BAP1 with subsequent impairment of the interaction between MLL3 and BAP1 by mutations of MLL3 PHD repeats of poor prognosis for cancer patients [25]. In our current work, we have detected exactly mutations that change the reading frame (p.Y816fs) and point mutations (p.D348N) KMT2CA. Mutations p.Y816fs previously described in colon cancer [26] and pancreatic neuroendocrine tumors [27]. However, bioinformatic analysis classified this mutation as benign especially that this variant

Table I. Mutations **detected in a studied case**

Gene ID	Ref seq	CDS	AA change	Allelic frequency (%)	Sequence depth	Pathogenecity
PARP1	NM_001618	c.2285T<C	p.V762A	62	462	Benign
RNF213	NM_001256071	c.3121G<A	p.A1041T	46	349	Benign
PAX8	NM_013992	c.404A>G	p.K135R	51	306	Pathogenic
MTRR	NM_024010	c.147A>G	p.I49M	66	659	Benign
KMT2C	NM_170606	c.1042G>A	p.D348N	12	723	Pathogenic
KMT2C	NM_170606	c.2447_2448insA	p.Y816fs	16	771	Benign

VUS – variant of unknown significance

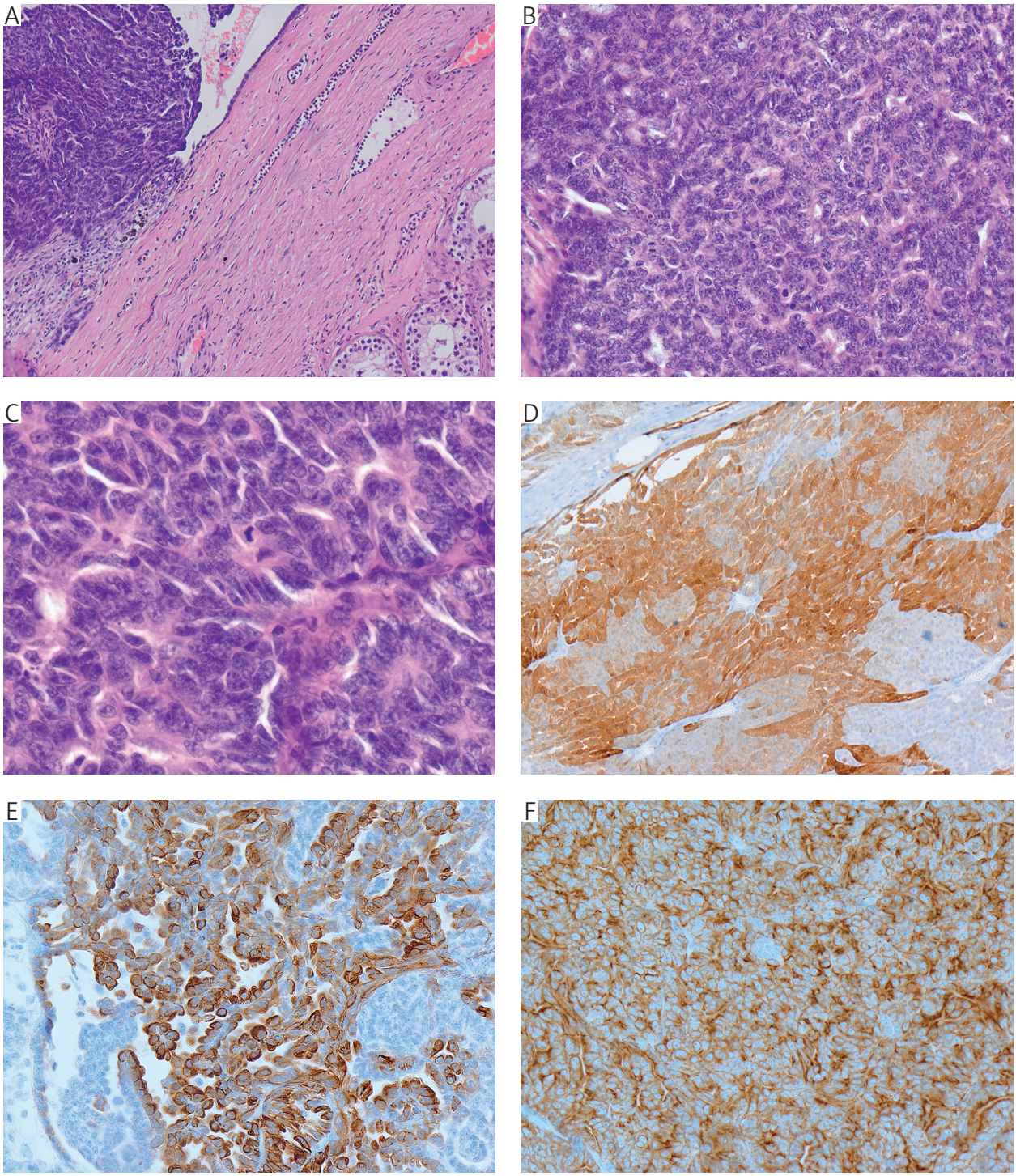

Fig. 1. Morphology and immunoprofile of malignant mesothelioma. A) Morphology of mesothelioma exophytic outgrowth at the visceral lamina of tunica vaginalis testis in this particular photo (HE, magnification 100×). B) Densely cellular texture of malignant mesothelioma with numerous mitoses (HE, magnification 200×). C) Frankly malignant cells of malignant mesothelioma (HE, magnification 400×). D) Mixed nuclear and cytoplasmic immunoreactivity to WT1 in malignant mesothelioma (WT1 staining, magnification 200×). E) Mixed membranous and cytoplasmic immunoreactivity to CK7 in malignant mesothelioma (CK7 staining, magnification 200×). F) Mixed membranous and cytoplasmic immunoreactivity to calretinin in malignant mesothelioma (calretinin staining, magnification 200×)

is very often detected in population (allelic frequency 0,48). In meanwhile, p.D348N mutations were detected in intestinal and pancreatic tumors, astrocytoma grade II and haemangioblastoma [27, 28, 29, 30]. To sum up, in the currently analyzed case, the molecular abnormalities significantly differ from the profile of mutated genes (*CDKN2A, NF2, BAP1, EGFR, NRAS*) described malignant pleural mesothelioma (MPM) [31, 32]. Although, we were not able to asses presence of homozygous deletion of *CDKN2A* with high confidence, looking at raw coverage data of the panel, we have noticed very low level of sequence depth (mostly less than 10 reads comparing to 137 reads [median]) of *CDKN2A* and *CDKN2B* locus. Considering 80% of tumor cells in the studied sample we suspect that both of *CDKN2A* alleles were lost in our case. Indeed, p16/CDKN2A is one of hallmark genes that get abrogated in diffuse malignant peritoneal mesothelioma (DMPM), so fluorescence *in situ* hybridization (FISH) detection of the homozygous deletion of p16/CDKN2A (p16) is an useful procedure for differential diagnosis of DMPM from reactive mesothelial hyperplasia (RMH), ovarian cancer and other malignancies that could spread to peritoneal surface [33].

Thus, the process of malignant transformation might develop in slightly different way and could be related to the anatomical location of the tumor. In spite of the numerous meticulous reports on morphology of malignant mesothelioma of testicular tunica vaginalis, there has been no molecular comprehensive research on gene profile in this rare malignancy so far [10, 34, 35]. Thus, our report certainly belongs to the first descriptions of the mutation profile of this category of tumors with some limitations as a single case study, as mesotheliomas present as truly heterogenous group of neoplasms in a variety of clinical settings [7, 36, 37, 38]. On the ground of our findings, development of MMTVT may be obscured by hereditary genetic predisposition in genes associated with DNA repair (PARP1), regulation of protein degradation (RNF213), surveillance of embryonic development (related to Müllerian duct origin) (PAX8), methionine synthesis (MTRR) and somatic changes linked to epigenetic regulation of gene expression (KMT2C). In our point of view, detection of mentioned mutations in MMTVT could be a ground for various, novel kinds of molecular targeted therapy, which have already been implemented in case of more frequent malignancies as colorectal cancer in case of anti-EGFR therapy that depends on KRAS and BRAF mutation status [39, 40, 41].

The authors declare no conflict of interest.

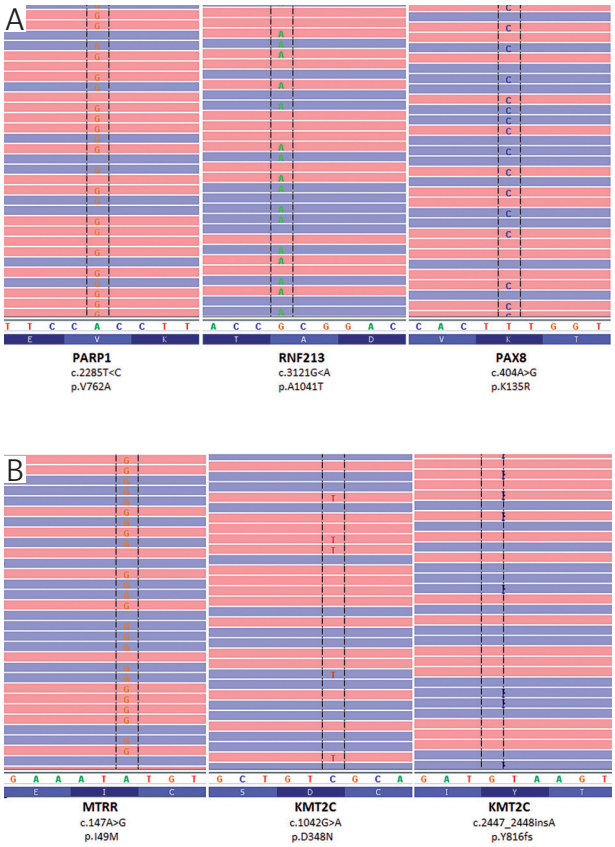

Fig. 2. Mutation detected by NGS in the studied case: sequencing results for PARP1 (p.V762V), RNF213 (p.A1041T), PAX8 (p.K135R), MTRR (p.I49M), KMT2C (p.D348N) and KMT2C (p.Y816fs) genes. Top: NGS data showing reads (− strand designated in blue, + strand in red) with the reference sequences shown below the panel boxes. Variant bases are indicated by green, red, brown and blue characters HE×100

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