

## CASE REPORT

**EXTRACARDIAC RHABDOMYOMAS – PRESENTATION OF TWO CASES WITH ANALYSIS OF AKT/mTOR AND ERK1/2 SIGNALING**EWA IŻYCKA-ŚWIESZEWSKA<sup>1</sup>, WOJCIECH WESOŁOWSKI<sup>2</sup>, BLANKA HERMANN<sup>1</sup><sup>1</sup>Pathology and Neuropathology Department, Medical University in Gdansk, Gdansk, Poland<sup>2</sup>PPL ELPAT in Elblag, Poland

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Extracardiac rhabdomyomas (RM) are very rare benign tumors with a poorly understood pathogenesis. In this report we describe two RM cases – a sublingual adult type tumor and a genital type tumor involving the uterine cervix. The patho-clinical characteristics, as well as the pioneer immunohistochemical analysis of ERK1/2 and AKT/mTOR pathway status is included. The expression of key proteins involved in above signaling gives new insight into the biology of extracardiac RM.

**Key words:** rhabdomyoma, cellular signaling, AKT/mTOR, extracardiac.

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**Introduction**

Rhabdomyomas (RM) are benign, rare lesions which account for about 2% of all tumors with skeletal muscle differentiation. They are divided into two main types: cardiac and extracardiac [1]. The cardiac one is a hamartomatous lesion, associated with a tuberous sclerosis complex (TSC). Cardiac RM occur in the children, exclusively in the heart, being often multifocal. Pathogenesis of this tumor is related to mutations of *TSC1* and *TSC2* genes, involved in regulation of cellular growth, proliferation and motility through mTOR signaling [1-4]. The extracardiac RM are defined as true neoplasms with a variable histology. Their clinical and radiologic presentation is unspecific and depends on the tumor location. The adult (about 50% of RM cases), fetal (40%) and genital (10%) subtypes are distinguished [1]. The treatment of choice of these tumors is a simple excision. However, RM can recur even many years after the primary excision, but without malignant transformation and metastases. The pathogenesis of extracardiac RM is unknown, while in few cases, SHH pathway activation and association with Gorlin's syndrome has been proved [5, 6].

The adult RM is the most common type, usually affecting the elder men. Its most common localization is the head and neck region, but other rare sites such

as mediastinum, skeletal muscle and esophagus were also reported [7-9]. The typical adult type localization suggests its origin from the branchial musculature of third and fourth branchial arches. Usually the lesion is solitary, less frequently polinodular or multifocal. Histological differential diagnosis includes granular cell tumor, hibernoma, paraganglioma, alveolar soft part sarcoma and reticulohistiocytoma [6, 9]. The fetal type RM divided into the classic, juvenile and fetal subtype, occurs mainly in the children, involving head and neck, with a preference for the postauricular region. The correct diagnosis of fetal type RM with myotube-like differentiation resembling rhabdomyosarcoma can be problematic [1, 5, 10]. Genital rhabdomyoma constitutes the rarest RM form, affecting lower female genital tract in the middle aged women. This lesion favors the vaginal region, less commonly vulva and extraordinarily uterine cervix. It forms a small polypoid mass, being usually asymptomatic or causing vaginal bleeding. In this case, other vulvo-vaginal spindle cell lesions should be excluded [11]. New, recently described category of RM represents its paratesticular sclerosing variant [12].

We present two RM cases including the adult and genital type, with the immunohistochemical analysis of AKT/mTOR and ERK1/2 signaling made for the first time. The activation of these pathways gives new insight into the pathogenesis of extracardiac RM.

## Material and methods

The tumor sections were formalin-fixed, paraffin-embedded and routinely stained with HE method. Next, the histochemical methods as: phosphotungstic acid hematoxin (PTAH), periodic acid Schiff (PAS), Gomori and Masson staining were carried out. The diagnostic immunophenotyping was performed with ready-to-use antibodies (DAKO, Denmark) detecting: vimentin, alpha smooth muscle actin (ASMA), caldesmon, desmin, MyoD1, Myf4, Cytokeratins: CKAE1/3, CK7, CK20, S100, CD68, Ki67, CD44. Signaling pathways analysis involved phosphospecific antibodies against p-Akt (Ser473, 736E11, 1 : 25), p-mTOR (Ser 2448, clone 49F9, 1 : 50), p-p70S6K (Thr 389, 1 : 100), p-4EBP1 (Thr 37/ 46, 236B4, 1 : 100) Cell Signaling Technology, MA, USA, and antibodies: pERK1/2 (ab 17942, 1 : 200), anti-tuberin [Y320] (ab32554) and anti-hamartin (ab32936) (1 : 100) (Abcam, UK). En Vision technology served for visualization, and diaminobenzidine as the chromogen. Appropriate positive and negative controls were carried out. Analysis of signaling pathways concerned the pattern of protein expression (membranous, cytoplasmic, nuclear) and its intensity (negative, low, strong) within the neoplastic cells.

## Case studies

**Case 1.** A 69-years old woman was referred to the Laryngological Department in April 2010, due

**Table I.** Immunophenotype of the tumors and the results of signaling pathway analysis

	ADULT RM	GENITAL RM
Vimentin	+++	+++
ASMA, caldesmon	negative	negative
Desmin, MyoD1, Myf4	+++	+++
Cytokeratins: AKAE1/3, CK7, CK20	negative	negative
S100	focal+	negative
CD68	negative	negative
Ki67 index	1,00%	0,00%
CD44	negative	negative
Bcl2	++	negative
PhosphoAKT	+++	++
Tuberin	+++	+++
Hamartin	+++	+++
Phospho mTOR	++	+++
Phospho 70S6K	+	negative
Phospho 4EBP1	+++	+++
Phospho ERK1/2	+++	+++

to the difficulties of speaking and chewing because of oral cavity tumor observed from several months. The physical examination disclosed a firm, painless sublingual mass covered by a normal mucosa. On the ultrasound exam the mass was solid, slightly hypoechogenic with a low blood flow. The gross total tumor excision in several pieces with a total diameter of about 3 cm was performed. In HE staining, the neoplastic tissue consisted of large, tightly packed polygonal cells with no mitotic activity, surrounded with a scant poorly vascularized stroma. The cells had peripherally located nuclei and abundant, eosinophilic granular or vacuolated cytoplasm. PTAH method visualized the cross striation, whilst PAS stain revealed glycogen in cytoplasmic vacuoles. The diagnosis supported with the immunophenotype was the adult type rhabdomyoma. The patient is free of symptoms in four years follow up.

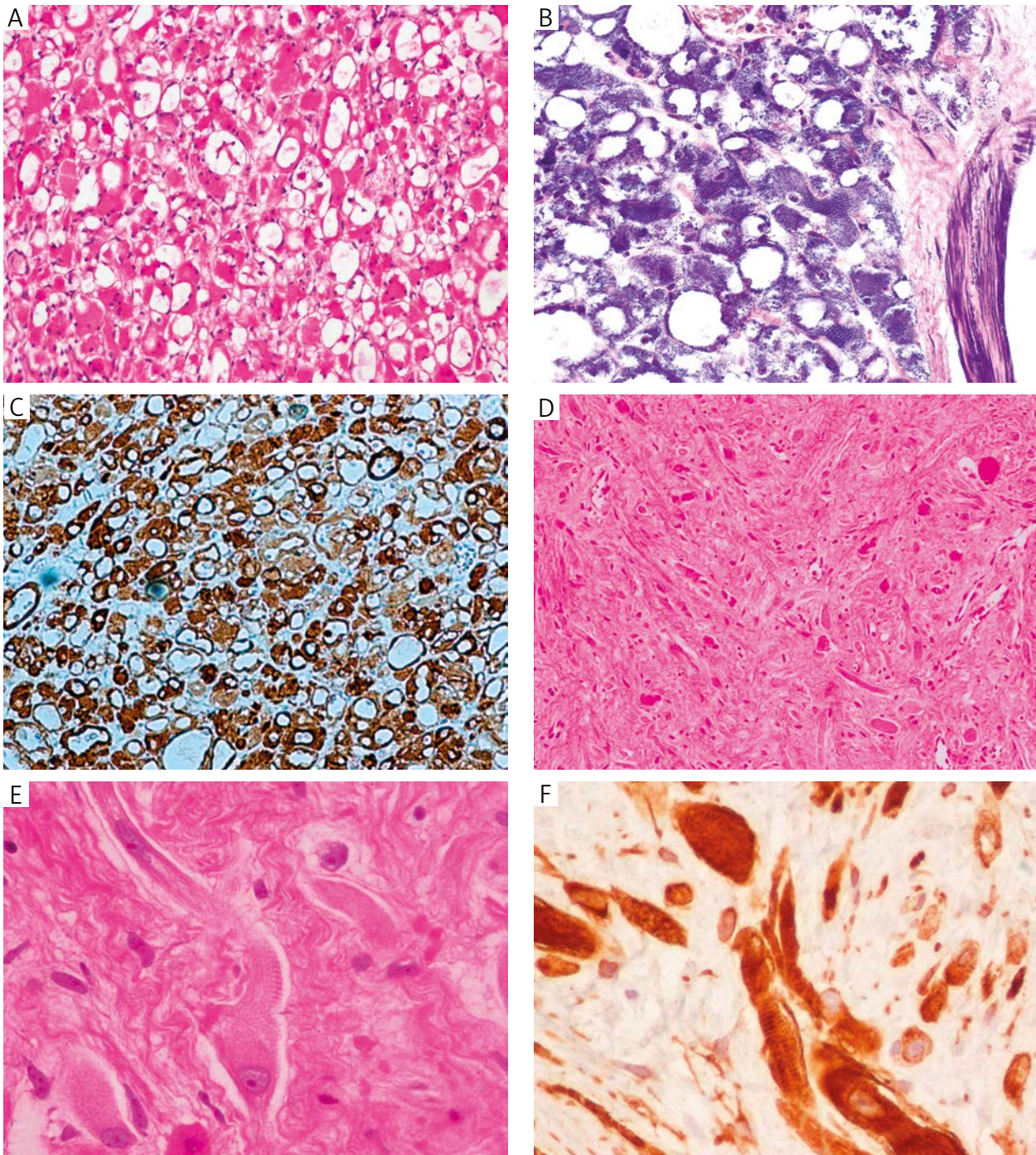
**Case 2.** A 50-years old women with a history of a recent slight vaginal bleeding was admitted to the Gynecological Department in June 2009. The examination disclosed the soft, elastic cervical polyp with a diameter of one cm, which was excised totally. Histologically, the polyp, covered with squamous epithelium, was composed of a dense, fibrous stroma with scattered atypical, spindle or polygonal cell without mitotic activity. The neoplastic cells formed fascicles and presented cytoplasmic cross-striation. The immunophenotype confirmed a diagnosis of a genital type rhabdomyoma. Five years follow up of the patient is uneventful.

## Signaling pathways analysis

Immunohistochemical examination disclosed activation of AKT/mTOR pathway in both tumors. Phospho AKT (p-AKT) positivity was found in the cytoplasm and nuclei of neoplastic cells, with a higher nuclear intensity in the adult RM. The expression of hamartin and tuberin was visible in cytoplasm and cellular membranes in both cases. Genital RM showed intense p-mTOR expression within a cytoplasm and nuclei, higher than the adult RM. P-70S6 kinase reactivity was found only in scattered nuclei of the adult, but not in genital case. Phospho 4EBP1 and p-ERK1/2 presented intense nuclear and cytoplasmic immunopositivity in both cases.

## Discussion

Rhabdomyomas together with rhabdomyomatosis of the lungs and rhabdomyomatous mesenchymal hamartoma, create a group of rare benign lesions with skeletal muscle differentiation [1]. In the last years the pathogenesis of cardiac RMs has been better understood through their association with tuberous sclerosis complex (TSC). This disorder, characterized by the formation of benign tumors and hamartomas

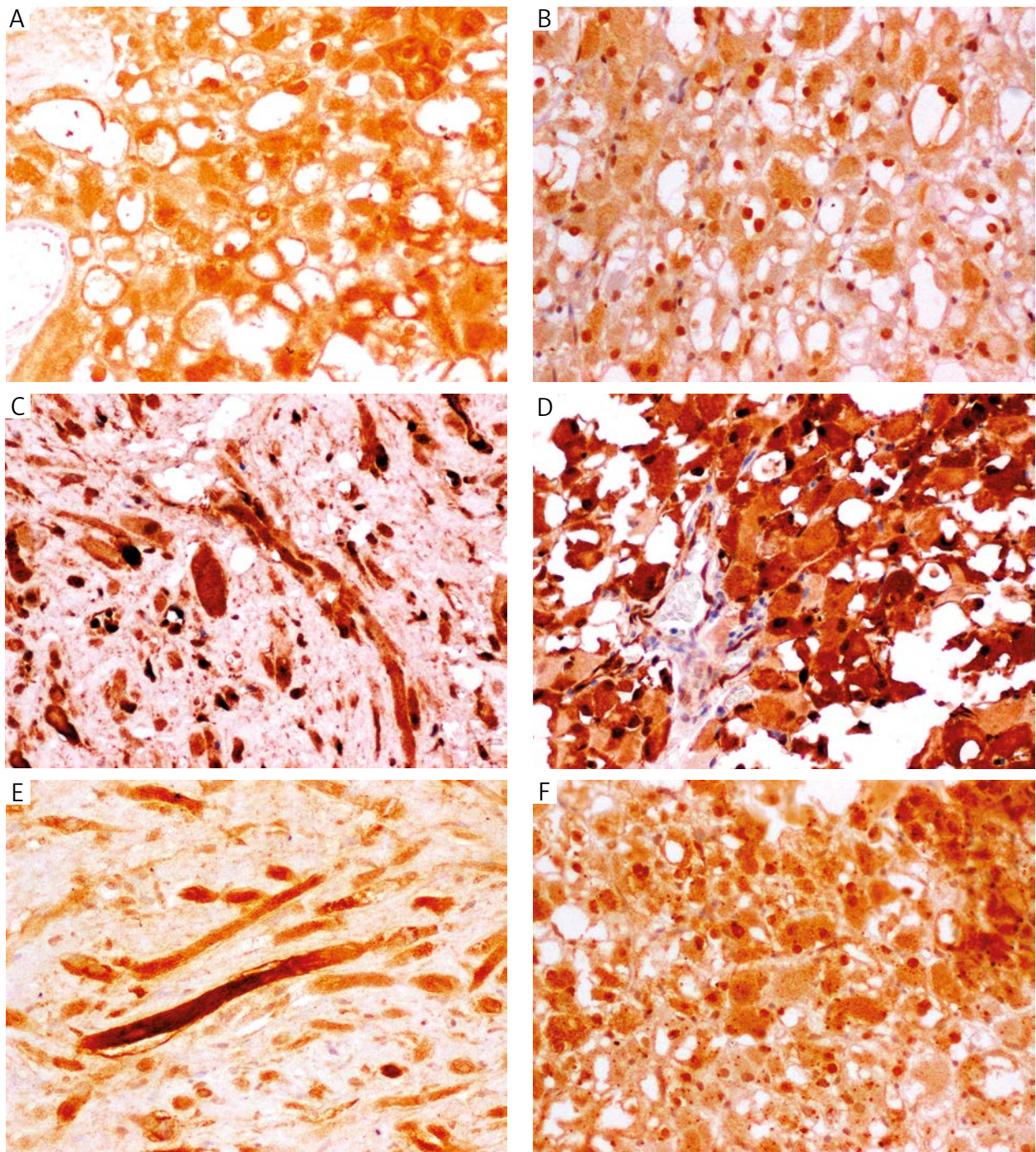


**Fig. 1.** Morphological features of the analyzed rhabdomyoma cases: A, B, C – adult RM; D, E, F – genital RM. A) Large polygonal and vacuolized cells with a granular, eosinophilic cytoplasm (HE, magnification 100 $\times$ ). B) The prominent cross-striation visualized in rhabdomyocytes with PTAH method (PTAH, magnification 200 $\times$ ). C) Intense desmin expression in the neoplastic cells (desmin, magnification 100 $\times$ ). D) The spindle neoplastic cells dispersed within the dense and fibrous stroma (HE, magnification 100 $\times$ ). E) The neoplastic cells characterized by vesicular nuclei and cytoplasmic cross-striation (HE, magnification 400 $\times$ ). F) Desmin-positive RM cells surrounded with the immunonegative stroma (desmin, magnification 400 $\times$ )

in multiple organs, is caused by *TSC* mutations affecting in loss hamartin and tuberin expression [4]. In a consequence, downstream constitutive activation of mTOR pathway up-regulates translation by p70S6K and 4E-BP1, contributing to the abnormal

cell growth and proliferation [2,3]. These findings have allowed to develop the target therapy with mTOR selective inhibitors for clinically problematic cardiac RM [4]. In normal condition, mTOR is activated by AKT kinase signaling through inhibition





**Fig. 2.** Signaling proteins analysis. A) High p4EBP-1 expression within the cytoplasm and neoplastic nuclei (magnification 200×). B) pAKT cytoplasmic and nuclear expression (magnification 200×). C, D) High nuclear and cytoplasmic ERK1/2 reactivity (200×). E, F) Intense pmTOR expression within the neoplastic cells (magnification 200×)

of TSC1/2 complex. Jozwiak et al. postulated, that an additional molecular trigger for TSC- associated tumors development is ERK potentiation [2]. The extracellular signal-regulated kinase ERK1/2 localizes in cytoplasm in active and inactive form, while activated, translocates to the nucleus to regulate the transcription [13].

Extracardiac RMs, subclassified into three pathological groups, have a poorly understood pathogen-

esis. Existence of clonal chromosome abnormalities in the adult RMs convince their neoplastic nature. The cytogenetic exam of one case showed reciprocal translocation of chromosomes 15 and 17 in 53% cells in metaphase and miscellaneous abnormalities in the long arm of chromosome 10 [8]. Single cases of fetal RM in the patients with Gorlin's syndrome (nevroid basal cell carcinoma syndrome) show Sonic Hedgehog (SHH) activation due to germline *PTCH*

mutation [5]. In mice, activated SHH signaling commonly induces tumors with myogenic differentiation. Finally, in few sporadic fetal and adult RM cases, SHH transduction cascade activation by unknown mechanism is reported [10]. However, no previous data on the other signaling, including AKT/mTOR pathway status in clinical samples of extracardiac RM are available.

Both presented here extracardiac RMs have a typical clinical and morphological characteristic for these unusual neoplasms, including a very rare case of cervical uterine genital tumor. We have found the activation of AKT/mTOR signaling in RM. Both tumors showed expression of p-AKT, p-mTOR, p-4EBP1, hamartin, tuberin, and in parallel high phospho-ERK1/2 reactivity. P70S6K kinase seems not to be involved in RM signal transduction, while 4EBP1 appears as the preferential translational effector. Some differences between the tumors occurred: bcl2 staining was detected in the adult case only, and p-mTOR expression was higher than p-AKT labeling in genital RM. AKT has connections with many transduction pathways and regulates apoptotic pathway and cellular proliferation. AKT-independent mechanisms of mTOR activation also exist, while both kinases are linked *via* positive and negative feedback loops [14-15]. In comparison to the extracardiac RM, in cardiac tumors, p-AKT upregulation was not detected, while mTOR and Erk, and their substrates were hyperactive [2, 3]. Moreover, hamartin and tuberin expression was decreased in cardiac tumors versus normal heart tissue. Finally, in a contrary to our genital case with antiapoptotic bcl2 expression, the upregulation of proapoptotic Bax protein in cardiac RM was disclosed [2, 3].

We describe for the first time activation of AKT/mTOR pathway, as well as ERK1/2, in extracardiac RM. The exact mechanism of this activation remains unestablished, if its background is genetic, epigenetic, or posttranslational. Probably coactivation of these pathways promotes tumorigenesis, being in parallel, involved in a myogenic differentiation [15]. Keeping in mind, that especially adult RM have relatively high propensity for recurrence, our findings suggest a potential way for their target therapy in the unresectable cases.

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*The authors declare no conflict of interest.*

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