Immunohistochemical mismatch in a case of rhabdomyoblastic metastatic melanoma

ADRIAN VASILE DUMITRU1,2), MIRCEA ŞTEFAN TAMPA3,4), SIMONA-ROXANA GEORGESCU3,4), STANA PĂUNICĂ5), CLARA NICOLETA MATEI3), ADRIANA ELENA NICA6), MARIANA COSTACHE1,2), ION MOTOFEI7), MARIA SAJIN1,2), IOANA PĂUNICĂ5), TIBERIU AUGUSTIN GEORGESCU1,2)

1)Department of Pathology, University Emergency Hospital, Bucharest, Romania
2)Department of Pathology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
3)Department of Dermatology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
4)Department of Dermatology, "Dr. Victor Babes" Hospital of Infectious and Tropical Diseases, Bucharest, Romania
5)Department of Periodontology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
6)Department of Anesthesiology and Intensive Care, University Emergency Hospital, Bucharest, Romania
7)Department of Surgery, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

Abstract
Melanomas can exhibit a wide range of unusual morphologies due to the neural crest origin of melanocytes. Several authors have documented variations in size and shape of cells, cytoplasmic features and inclusions, nuclear features and cell architecture. Metastatic melanoma with rhabdomyoblastic differentiation is an extremely rare condition with poor prognosis. Few studies concerning rhabdoid or rhabdomyoblastic differentiation in melanoma are currently available and the current report highlights some of the most important immunohistochemical features of this rare entity. We report on a case of a rhabdomyoblastic metastatic melanoma showing intense positivity for both melanocytic and rhabdoid markers in two cell populations dissociated within the tumor with multiple mismatches in immunomarker expression. Improved recognition of this rare morphological pattern may provide the means for developing new techniques to identify novel therapeutic targets, which would improve the prognostic outlook for these patients.

Keywords: rhabdomyoblastic, melanoma, metastasis, prognosis, immunohistochemistry.

Introduction
Melanomas can display variable histopathological features, especially in metastatic disease. True rhabdomyoblastic differentiation in metastatic melanoma is very rare [1–4] and should not be confused with the rhabdoid cell morphology that can sometimes be seen in melanoma, regardless of the primary tumor site. Rhabdomyoblastic differentiation in primary or metastatic melanoma is distinct from rhabdoid melanoma. In this morphological version, malignant cells are similar to rhabdomyoblasts because of the cytoplasmic aggregation of intermediate filaments and peripherally placed nuclei. Rhabdoid melanoma, however, lacks expression of striated muscle distinct markers such as myogenin, MyoD1, and myoglobin [2, 4–6].

Malignant melanoma with rhabdomyoblastic differentiation tends to occur in middle-aged to older patients and it seems there is no apparent gender predilection [7, 8].

Unusual melanoma variants can be problematic in terms of correct histopathological assessment and are a potential diagnostic pitfall especially in patients with unknown primary tumors or in cases with limited access to patient history. The scale of uncommon phenotypes, which can be seen in melanoma, encompass the expression of morphotypes, biomarkers and antigens frequently encountered in lesions of epithelial, fibrohistiocytic, rhabdoid, smooth muscle, and osteo-cartilaginous origin [2–5, 8, 9]. On the other hand, there are some melanomas that do not express biomarkers related to melanocytic lesions, such as S100 protein or human melanoma black-45 (HMB-45), especially in metastatic disease [10]. Thus, cases like these turned out to be particularly deceptive diagnostic pitfalls.

The aim of this study was to identify and characterize some peculiar immunohistochemical (IHC) features in a case of metastatic melanoma and to explore a novel approach to better recognize true rhabdomyoblastic transdifferentiation.

Case presentation
P.M., a 57-year-old woman, was admitted at the Emergency University Hospital in Bucharest (Romania), on November 10, 2016, accusing abdominal pain, upper gastrointestinal bleeding, nausea and vomiting. At first glance, her past medical history was unremarkable. Laboratory data revealed a moderate anemia and a slight leukocytosis. Gastroenterological examination, including endoscopy and colonoscopy, was normal. A computed tomography (CT) scan with i.v. contrast suggested a 5×4×4cm tumor with considerable enhancement, located in the endopelvic portion of the ileum, associated with bulky pelvic and para-aortic lymphadenopathy. At this
point, the patient underwent an emergency surgical intervention in the same clinic, followed by histopathological examination. The *in situ* gross aspect of the tumor was suggestive for a gastrointestinal stromal tumor (GIST), but the subsequent frozen section examination confirmed this initial diagnosis and conventional examination of the surgical samples revealed a malignant proliferation with peculiar rhabdoid-like cells.

After surgery, upon further investigation of the personal medical history of our patient and we found that she previously underwent multiple surgical interventions in a different medical unit, in order to excise several tumor masses from her cervical region, liver, brain and skin, which eventually turned out to be metastases of melanoma. The primary site of the tumor was unknown. The patient deceased four days after the last surgical intervention due to cardiovascular comorbidities.

Upon gross examination, the large ileal tumor indeed resembled a well-circumscribed GIST with a cut tan-gray surface, showing areas of infarction, hemorrhage and necrosis (Figure 1). Specimen samples were fixated with 10% buffered formalin and were processed by conventional histopathological methods using paraffin embedding, sectioning and Hematoxylin–Eosin (HE) staining. Histopathological examination revealed a morphologically heterogeneous malignant proliferation, with two main different types of cells: some characteristic for melanoma and others showing rhabdoid features (Figure 2).

The tumor was penetrating the serosa, invading the underlying intestinal wall (Figure 3). The rhabdoid cells had eosinophilic hyaline-like perinuclear inclusions; compressed, eccentric nuclei; prominent central macronuclei; abundant eosinophilic cytoplasm and reticular chromatin pattern (Figure 4). Both conventional microscopic examination and Fontana–Masson staining did not reveal any melanin pigment. At the same time, we also reexamined the previous slides from the other metastatic sites. All specimens revealed classical microscopic aspects of melanoma: atypical melanocytes with marked cytological atypia and no maturation, which invade the surrounding tissue, large cells with abundant eosinophilic and finely granular cytoplasm, pleomorphic nuclei with large eosinophilic nucleoli and occasional nuclear pseudoinclusions, folds or grooves. All previous metastases were heavily pigmented.
Since we considered this shift in morphology interestingly odd, we performed ancillary tests in order to bring light to this peculiar change. To begin with, the paraffin blocks were sliced at microtome and the resulting 3-μm thick sections were mounted on slides covered with poly-L-Lysine. After that, the sections were deparaffinized in toluene and alcohol successive baths, one hour (15 minutes by bath), rehydrated (three successive alcohol baths with decreased concentration: 96%, 80% and 70%, followed by a bath with distillate water for 10 minutes). The slides were washed in phosphate-buffered saline (PBS), incubated with normal serum for 20 minutes and after with the primary antibody over-night, using Dako labeled Streptavidin–Biotin (LSAB) kit. The next step consisted in carbonate buffer washing and development in 3,3’-Diaminobenzidine (DAB) hydrochloride/hydrogen peroxide. The last step included nuclear counterstaining with Mayer’s Hematoxylin. We used the following antibodies from Biocare: S100 (mouse monoclonal, clone 15E2E2 + 4C4.9, 1:100 dilution), epithelial membrane antigen (EMA) (mouse monoclonal, clone E29, 1:100 dilution), CD31 (rat monoclonal, clone MEC13.3, 1:50 dilution), CD34 (mouse monoclonal, clone QBEnd/10, 1:100 dilution), smooth muscle actin (SMA) (mouse monoclonal, clone 1A4 – also known as asm-1, 1:100 dilution), Melan A (mouse monoclonal, clone A103, 1:25 dilution), HMB-45 (mouse monoclonal, clone HMB-45, 1:100 dilution), Ki67 (mouse monoclonal, clone MIB-1, ready-to-use), desmin (mouse monoclonal, clone D33, 1:50 dilution), myogenin (mouse monoclonal, clone MyG007 – also known as F5D, 1:100 dilution), MyoD1 (rabbit monoclonal, Abcam clone, 1:200 dilution), vimentin (mouse monoclonal, clone V9, 1:200 dilution), DOG-1 (mouse monoclonal, clone DOG1.1, 1:100 dilution), and CD117/c-Kit (rabbit monoclonal, clone EP10, 1:50 dilution).

IHC tests showed intense positivity for both melanocytic and rhabdoid markers. The tumor cells demonstrated unique immunophenotypic heterogeneity. There were two cell populations dissociated within the tumor, and in some areas, we observed a gradual shift in morphology from classic melanoma to rhabdoid-type cellularity. The immunostainings showed a clear demarcation of those areas with many mismatches in immunomarker expression. PanMelan cocktail (Figure 5), S100 (Figure 6) and melanoma-associated antigen recognized by T cells-1 (MART-1) (Figure 7) were positive in the classic melanoma component, whereas the rhabdoid part was negative or showed little expression. HMB-45 was negative in both components (Figure 8).
CD117 and DOG-1 were also negative, excluding a GIST. Interestingly, Ki67 expression was lower in the rhabdoid areas of the tumor (Figure 9). CD31 and CD34 showed marked neoangiogenesis especially in the rhabdoid component (Figure 10). Vimentin expression was positive in both components but was more prominent in the areas of classic melanoma (Figure 11). Desmin (Figure 12) and actin (Figure 13) were highly expressed in the rhabdoid part of the tumor. Myogenin (Figure 14) and MyoD1 were almost exclusively expressed in the rhabdoid areas, which demonstrated that those cells are not only rhabdoid cells, but they have true rhabdomyoblastic differentiation. The final histopathological diagnosis was intestinal metastatic amelanotic melanoma with rhabdomyoblastic transdifferentiation.
Discussions

Melanomas can exhibit a broad spectrum of different histomorphologies, mainly due to their neural crest origin. Cellular and nuclear pleomorphism is one of the key features seen in melanoma along with occasional non-melanocytic differentiation such as schwannian, nevoid, neur oid, fibroblastic, myofibroblastic and, in rare cases, even squamous, osteocartilaginous and ganglionic differentiation [3, 11]. Melanoma, like several other tumor types, occasionally demonstrates a rhabdoid phenotype [5, 12, 13], but in the majority of those cases only the morphology evokes these features as the cells are not true rhabdomyoblasts. True rhabdomyoblastic transdifferentiation is extremely rare and can be a real challenge even for an experienced pathologist.

Rhabdomyoblastic differentiation is described in a very small subgroup of rhabdoid tumors and demands specific immunohistochemical expression of striated muscle-related markers, genes or proteins or additional electron microscopic evidence of striated muscle transdifferentiation [2, 6]. Rhabdomyoblastic differentiation in melanoma is extremely rare, and as far as we know there are only seven previously reported cases in the scientific literature, but in none of those, the shift in morphology and immunoexpression is simultaneously present in the same tumor site. Our case shows this phenomenon best, as demonstrated by the many immunohistochemical mismatches between the classic melanoma component and the adjacent rhabdoid area.

The first case report of rhabdomyoblastic differentiation in melanoma [14] is questionable as it presents a case of a cerebral melanoma metastasis with this rare feature based only on cell morphology, with no additional ancillary studies. Pasz-Walczak et al. reported two cases [1], both with loco-regional metastases. One of these cases has similar immunophenotype with our case, expressing S100, vimentin, and desmin but was negative for HMB-45. Is to be noted that HMB-45, a very specific marker for melanoma, may be lost in the metastatic deposits. Although the above-mentioned authors showed that the metastases revealed some sarcomatous features, they did not extend the research by using specific striated-muscle markers. Another two cases were reported by Gharpuray-Pandit et al., one in a patient with metastatic disease, with no known primary tumor site [2]. This time, the authors used specific striated muscle immunomarkers, such as myogenin, myoglobin and MyoD1 and observed some scattered tumor cells positive for those markers, thus supporting the theory that these manifestations are a faulty or imperfect development of the neural crest with eventual mesenchymatous transdifferentiation [17–19].

Despite the scarcity of data and low number of case reports in the scientific literature, it is extremely difficult to extrapolate prognostic information and the incidence of regression or apoptosis. However, rhabdoid tumors are usually aggressive and associated with a relatively poor prognosis [18–20].

As in all fields of Pathology, the paradigm for the therapeutic management of melanoma is shifting towards mutational profiling and elaboration of targeted treatments for the most important etiological factors in melanoma development and progression. Second histopathological opinion is often recommended as well as referral to a specialized unit for discussion in a multidisciplinary setting.

Conclusions

This case report emphasizes the importance for both clinicians and pathologists to have a high index of suspicion regarding malignant melanoma in metastastic disease with uncommon morphology and/or immunophenotype. In patients with personal history of malignant melanoma and a newly diagnosed rhabdomyosarcoma, differential diagnosis should always be taken into consideration. Moreover, we highlight the need for extensive information regarding differentiation patterns to assist the development of targeted therapies for specific histopathological subtypes of melanoma.

Conflict of interests

The authors declare that they have no conflict of interests.
Ethical standards
We undersign, certificate that the procedures and the experiments we have done respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2000, as well as the national law.

References

Corresponding author
Stana Păunica, Associate Professor, DMD, PhD, Department of Periodontology, “Carol Davila” University of Medicine and Pharmacy, 37 Dionisie Lupu Street, Sector 2, 020022 Bucharest, Romania; Phone +40745–998 304, Fax +4021–318 07 30, e-mails: spaunica@yahoo.com, stana.paunica@umfcd.ro

Received: December 15, 2017
Accepted: May 5, 2018