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Case Report

Cancer Cell Plasticity in Urothelial Carcinoma: When it Give Rise to Melanoma

Alix Fontaine[1], Denis Roblet[2], Racha Benmeziani[3], Gaelle Fromont[1,4*]

[1]Department of Pathology, Bretonneau Hospital, University Hospital - University of Tours, 37000 Tours, France

[2]Department of Pathology, Saint Michel Hospital, 16959 Angouleme, France

[3]Department of Urology, Saint Michel, 16959 Angouleme, France

[4]Inserm UMR U1069, 37000 Tours, France

[*]Corresponding Author: Gaelle Fromont, Department of Pathology, Bretonneau Hospital-CHU de Tours, 2 Bd Tonnellé, 37044 Tours Cedex 9, France.

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Abstract

Primary bladder melanoma is very rare, and the association with urothelial carcinoma has never been described to date. We report a case of a biphasic tumor of the bladder, composed of high grade urothelial carcinoma adjacent to melanoma, characterized using immunohistochemistry and DNA sequencing. The urothelial component was positive for pan-cytokeratin and GATA3, and negative for melanoma markers HMB45, Melan A, and Sox10. In contrast, the melanocytic component was negative for pan-cytokeratin and positive for HMB45, Melan A, and Sox10. Moreover, some nuclei were weakly stained for GATA3, a transcription factor of the urothelial lineage. Molecular genetic analysis showed that both components shared a never described identical *serine/threonine kinase 11* (*STK11)* D365G molecular alteration, and a c.-124C>T *Telomerase Reverse Transcriptase* (*TERT)* promoter mutation, an early event in urothelial carcinogenesis. Our report underlines the phenotypic plasticity of urothelial

carcinomas, and raises the question of an urothelial origin of urinary tract melanomas.

Keywords: Cancer cell plasticity; Cancer stem cell; Melanoma; Urothelial carcinoma; Bladder

1. Introduction

The juxtaposition of two tumors with distinct morphology and immunophenotype is relatively rare, and could be induced by either collision tumors or transdifferentiation of a malignant component into a different phenotype. Collision tumors are two independent neoplasms, with different cells of origin, that occur in the same anatomic site. In contrast, transdifferentiation of one component to another or divergent differentiation from a cancer stem cell population implies a common origin. We described herein a case of bladder melanoma associated with high grade urothelial carcinoma, and evidenced a filiation between the two components using molecular genetic analysis.

2. Case Report

A 80-years-old man without previous history of urothelial tumor or melanoma, underwent a transuretral resection of the bladder for gross hematuria. Both clinical examination and Positron Emission Tomography (PET) ^{18}F-fluorodeoxyglucose(^{18}F-FDG) performed after the diagnosis revealed no other cancer localization. Microscopic analysis showed a biphasic tumor invading the bladder wall, with adjacent components. The most representative component (A) was composed of large size cells, with eosinophilic cytoplasm, irregular nuclei, and numerous mitoses. Cells were arranged in clusters or nests within a fibrous stroma (Figure 1A). The second component (B) was composed of sheets of smaller cells (Figure 1B), without pigment deposition. Cells of the component A were positive after staining for pan-cytokeratin, GATA3 (Figure 1C), and were negative for HMB45 (Figure 1E), Melan A, and Sox10 (Figure 1G). In contrast, component B was negative for pan-cytokeratin, and was diffusely positive for HMB45 (Figure 1F), Melan A, and Sox10 (Figure 1H). In addition, few nuclei were weakly stained for GATA3 (Figure 1D). No expression was found for the neuroendocrine markers Chromogranin A and Synaptophysin. Moreover, we observed an overexpression of P53 in both components (Figure I and J). Samples of both components were macrodissected from the paraffin blocks, and analyzed with Next Generation Sequencing (NGS). NGS analysis identified similar genetic alterations in both components for *serine/threonine kinase 11* (*STK11)* and *telomerase reverse transcriptase* (*TERT)*. Moreover, a mutation of *phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA)* was observed in the urothelial component (Table 1). No anomalies were found in the following genes: *AKT1, ALK, BRAF, CTNNB1, DDR2, EGFR, ERBB2, ERBB4, FGFR2, FGFR3, HRAS, KIT, KRAS, MAP2K1, MET, NRAS, PDGFRA, POLE, and RAC1*. These findings led us to propose the final diagnosis of high-grade urothelial carcinoma with focal melanocytic transdifferentiation. Due to his age and comorbidities, the patient was planned to be treated by radiation therapy using 36 Gy in 6 fractions. The patient died 2 months after diagnosis.

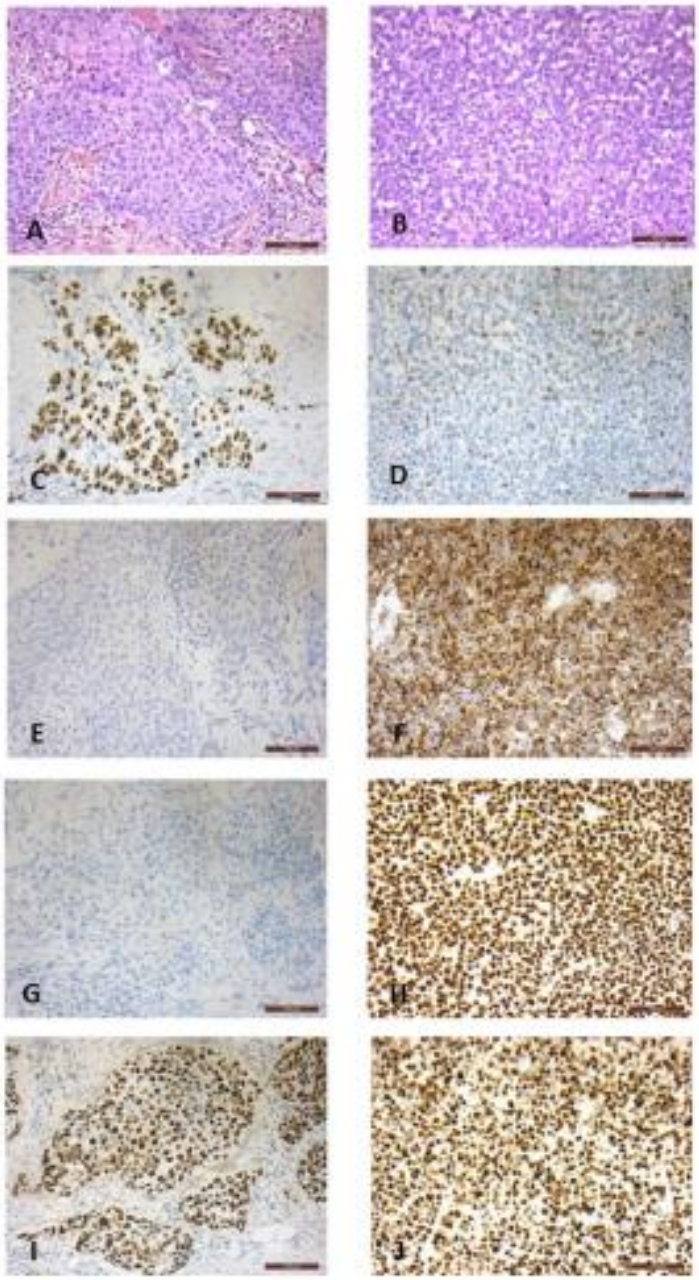

Figure 1: HES staining and immunohistochemical findings in both components. The most representative component (A) is composed of large or intermediate size cells, with eosinophilic cytoplasm, enlarged and irregular nuclei, and numerous mitoses. Cells are arranged in clusters or nests within a fibrous stroma (HES, A). The second component (B) is composed of sheets of smaller cells (HES, B). Cells of the component A are positive after immunohistochemical staining for GATA3 (C), and negative for HMB45 (E), and Sox10 (G). In contrast, component B is diffusely positive for HMB45 (F) and Sox10 (H), with few nuclei weakly stained for GATA3 (D). P53 protein was overexpressed in both components (I, J).

Component	Gene	Variant	Protein alteration	Significance
Melanoma	STK 11 exon 8	c.1094A>G	p.(Asp365Gly)	Uncertain significance
	TERT promoter	c.-124C>T		Pathogenic
High-grade urothelial carcinoma	STK 11 exon 8	c.1094A>G	p.(Asp365Gly)	Uncertain significance
	TERT promoter	c.-124C>T		Pathogenic
	PIK3CA exon 9	c.1624G>A	p.(Glu542Lys)	Pathogenic

Table 1: Genetic alterations in the melanoma and high-grade urothelial carcinoma components.

3. Methods

Immunohistochemistry was performed with the automated BenchMark XT stainer (Ventana Medical Systems Inc., Oro Valley, AZ) using OptiView Detection Kit (Ventana Medical Systems Inc.). Slides were deparaffinized, rehydrated, and heated in citrate buffer pH 6 for antigenic retrieval. The primary antibodies included CK AE1/AE3 (AE1/AE3 clone, 1/200 dilution, 32 min; Dako, Carpintera, CA), GATA3 (L50-823 clone, 1/1 dilution, 32 min at 37°C; Ventana, Rotkreuz, Switzerland), HMB45 (HMB45 clone, 1/50 dilution, 16 min at 37°C; Dako), Melan A (A103 clone, 1/1 dilution, 16 min at 37°C; Ventana), Sox10 (SP267 clone, 1/1 dilution, 32 min at 37°C; Ventana), Chromogranin A (CK2H10 clone, 1/200 dilution, 60 min; Zytomed, Berlin, Germany), Synaptophysin (DAK-SYNAP clone, 1/50 dilution, 32 min; Dako), and P53 (D07 clone, 1/50 dilution; 32 min, Dako). Tumor DNA was extracted after macrodissection of formalin-fixed paraffin-embedded tissue sections for each contingent, using a Maxwell® 16 FFPE Tissue LEV DNA Purification Kit (Promega®, Madison, WI), according to the manufacturer's instructions, and a Maxwell® 16 instrument (Promega®). DNA concentration was assayed using a Qubit 4 fluorometer (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. The sequencing libraries were prepared using a QIAseq Targeted DNA Custom Panel kit (amplicon technology, Qiagen®, Germantown, MD), following the supplier's recommendations. Sequencing was performed on an Illumina Miseq Platform. Bioinformatic analysis was performed with an internal pipeline, generated using CLC Genomics Workbench software (Qiagen®).

Sequence alignment was performed using the GRCh37 (hg19) genome. List of targeted genes, exons and mRNA RefSeq used (NM) : *AKT1* (exon 3, NM_001014431.1), *ALK* (exons 20, 21, 22 23,24 and 25, NM_004304.4), *BRAF* (exons 11 and 15, NM_004333.4), *CTNNB1* (exons 2 to 4 and 6 to 8, NM_001904), *EGFR* (exons 18, 19, 20 and 21, NM_005228.4), *ERBB2* (*HER2*)(exon 20, NM_004448.3), *ERBB4* (exons 10 and 12, NM_005235.2), *FGFR2* (exons 7, 12 and 14, NM_000141.4), *FGFR3* (exons 7, 9 and 14, NM_000142.4), *HRAS* (exons 2, 3 and 4, NM_005343.3), *KIT* (exons 8, 9, 11, 13, 17 and 18, NM_000222.2), *KRAS* (exons 2, 3 and 4, NM_004985.4), *MAP2K1* (exon 2, NM_002755.3), *MET* (exons 2 and 14 à 20, NM_001127500.2), *NRAS* (exons 2, 3 and 4, NM_002524.4), *PDGFRA* (exons 12, 14 and 18, NM_006206.5), *PIK3CA* (exons 9 and 20, NM_006218.3), *POLE* (exons 9 to 14, NM_006231) *RAC1* (exon 2, NM_018890), *STK11* (exons 1 to 9, NM_000455), *TERT* (promoter).

4. Discussion

This association of two components in a patient without any cancer history suggests either a collision of primary bladder melanoma with urothelial carcinoma, or a filiation between the two components. To date, less than 50 primary bladder melanomas have been reported, whereas urothelial carcinoma is the most frequent bladder cancer [1]. The second hypothesis is supported by both immunohistochemical and genetic findings. The focal expression of GATA3 in the melanocytic component of the tumor is in favor of a filiation between the urothelial origin and the melanocytic differentiation. Moreover, NGS analysis identified similar genetic alterations in both components for *STK11* and *TERT*. The tumor suppressor *STK11* encodes a member of the serine/threonine kinase family that activates AMPK and negatively regulates mTOR signaling pathway. *STK11* point variations are common in urothelial carcinoma, particularly in high grade cancer, where it has been shown to activate mTOR pathway [2]. In skin metastatic melanoma, *STK11* is mutated in around 15% of cases [3]. However, *STK11* p.(Asp365Gly) alteration has never been described in either urothelial carcinoma or melanoma. Its significance remains uncertain, but the finding of this specific genetic alteration in both the urothelial and melanocytic component of the present case is in favor of a filiation. Reactivation of telomerase activity drives human cell immortality and cancer [4]. In urothelial carcinoma, telomerase activity level correlates with pathologic grade and clinical stage [5], and has been suggested to be an early event in bladder carcinogenesis [6]. Point variations of the *TERT* promoter occur in more than 50% of urothelial carcinoma, with the c.-124 nucleotide involved in the majority of cases, but none of them was a c.-124C>T variation as in our case [7]. Around 80% of metastatic melanomas have *TERT* promoter variations, and one of the hot spots is the c.-124C>T [2,7]. Although *TERT* promoter variations have been shown to occur often together with *BRAF* or *NRAS* variations, none of these genes was mutated in the present case. One previous report has analyzed the genetic alterations in melanomas of the urinary tract, and evidenced in only 2 of 8 cases BRAF p.(Val600Xxx) variations [8]. We observed in the urothelial component a variation of *PIK3CA* that was not found in the melanocytic component. *PIK3CA* is involved in the PI3K signaling pathway, as *STK11*. *PIK3CA* variations have been described in around 10% of urothelial cancers [9], and some of them led to alteration of the protein p.(Glu542Lys), as in our case. Similar genetic alterations have been used to evidence a filiation between two cancer components. Rearrangements involving *ERG* and the androgen regulated gene *TMPRSS2*, specific of prostatic adenocarcinoma, have also been evidenced in small cell neuroendocrine prostate cancers, suggesting that neuroendocrine cancer cells in this location are derived from adenocarcinoma cells [10]. In small cell neuroendocrine carcinoma of the bladder, the urothelial origin is supported by the finding of *TERT* promoter variations, common in urothelial carcinomas, but not observed in small cell neuroendocrine carcinomas from other locations [11]. The transformation of a cancer component into another component with a different phenotype and different cell lineage characteristics is called phenotypic plasticity. It could be due to transdifferentiation, *i.e.* conversion of a differentiated cell into another differentiated cell of distinct lineage, or to divergent differentiation from a Cancer Stem Cell (CSC) population. Transdifferentiation and divergent differentiation shares common mechanisms, since it has been shown that differentiated cancer cells can acquire CSCs properties, allowing the tumor to adapt quickly to its environment [12]. These mechanisms are those involved in the Epithelial-to-

Mesenchymal Transition process, that contributes to tumor progression and aggressiveness [12]. Both urothelial carcinoma and melanoma have been shown to have propensity for divergent differentiation. The range of divergent differentiation in melanoma include most often mesenchymal or neuroendocrine, and rarely epithelial differentiation [13]. Several types of variants have been described in urothelial carcinomas, including squamous, glandular or neuroendocrine differentiation [14,15]. Due to the rarity of primary bladder melanoma, it is likely that in the present case the cancer component of origin is urothelial carcinoma. In conclusion, the present report provides pieces of evidence for a filiation between high grade urothelial cancer and bladder melanoma, that has never been described to date, and underlines the phenotypic plasticity of urothelial carcinomas.

Compliance with Ethical Standards

All experiments have been performed for diagnostic purpose. Studies are conducted according the guidelines of the local ethical committee.

Source of Funding

None

Conflict of Interest

The authors have no conflict of interest to declare

Contributions

Alix Fontaine (junior pathologist), data analysis, wrote the manuscript

Denis Roblet (local pathologist), provided biological ressources

Racha Benmeziani (Urologist) in charge of the patient, performed the resection

Gaëlle Fromont (senior pathologist): design of the project, data analysis, wrote the manuscript

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