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Letter to editor

Mutational profiling of *SMARCA4* and *SMARCB1* in ampullary adenocarcinoma: a preliminary study

Paola Parente[1], Francesco Pepe[2], Claudia Covelli[3], Gianluca Russo[2], Federica Russo[1], Giancarlo Troncone[2], Paolo Graziano[1,4*], Umberto Malapelle[2*]

[1] Pathology Unit, Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo (FG), Italy; [2] Department of Public Health, Federico II University of Naples, Naples, Italy; [3] Pathology Unit, Ospedale Desenzano del Garda, Italy; [4] Department of Radiological, Oncological and Pathological Sciences, Sapienza University of Rome, Rome, Italy

**Co-Last Author*

Dear Editor,

Ampullary adenocarcinoma (AAC) accounts for only 0.2% of gastrointestinal malignancies and, although a surgical approach is suitable in 80% of cases, the 5-year survival rate is 30-50% with a high recurrence rate (50%) [1]. The most frequent histological subtypes are mixed, pancreatobiliary (PB), and intestinal (INT) [1]. Therapeutic approaches are limited, with approved gemcitabine-based regimen in PB and 5-fluororacil in INT AAC patients, respectively [1]. AAC molecular profiling predominantly highlighted *KRAS* mutations followed by *TP53*, *WNT/APC*, *PIK3CA*, *SMAD4* then *BRAF*, *CDKN2A*, *FBXW7*, *ERBB2*, *PDFGRA*, *SMARCB1*, *RB1* [1]. Among them, only *BRAF* point mutations (detected in 9.3% of cases) and NTRK aberrant transcripts (2.0% of cases) make AAC patients eligible for Idabrafenib plus trametinib (*MEK* inhibitors) and anti *NTRK* inhibitors, respectively [1]. In this scenario, the identification of novel specific molecular hallmark able to optimize the clinical management of AAC patients is crucial. *SMARC* proteins are core subunits of the SWI1/SNF1 complex and are involved in chromatin remodeling and DNA repair [2]. *SMARCA4* (BRAHMA RELATED GENE, *BRG1*), involved in *ERBB*, *GnRH*, and *PI-3K*/*AKT*/*mTOR* signaling pathways, acts in transcriptional modulation, DNA repair, and cell cycle checkpoint [2]. A high expression level of *SMARCA4* has been described as a negative prognostic factor across different solid tumors [2]. A plethora of *SMARCA4* molecular alterations, including missense mutations, indels and copy number variations (CNVs), were identified [2].

SMARCB1 (integrase interactor 1, *INI1*), involved in SWI1/SNF1 complex, plays a crucial role in tumor suppression through the epigenetic mechanisms [3]. Moreover, *SMARCB1* is directly involved in downregulating *INK4A, WNT*-signaling pathway, repressing retinoblastoma (*RB*) target genes and promoting *MYC* oncogene-mediated transactivation [3]. *SMARCB1* molecular alterations, depending on biallelic inactivation from deletions, large intragenic deletions/duplications, small out-of-frame intragenic deletion/insertions, splice-site, nonsense and truncating mutations, lead to the complete loss of function in malignant neoplasms [3]. Interestingly, rare *SMARCB1* missense mutations were identified in sporadic AAC cases, without any details about the molecular landscape [4].

Here, we aimed to overview the preliminary results from *SMARCA4* and

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Correspondence
Francesco Pepe
E-mail: pepefrancesco88@yahoo.it

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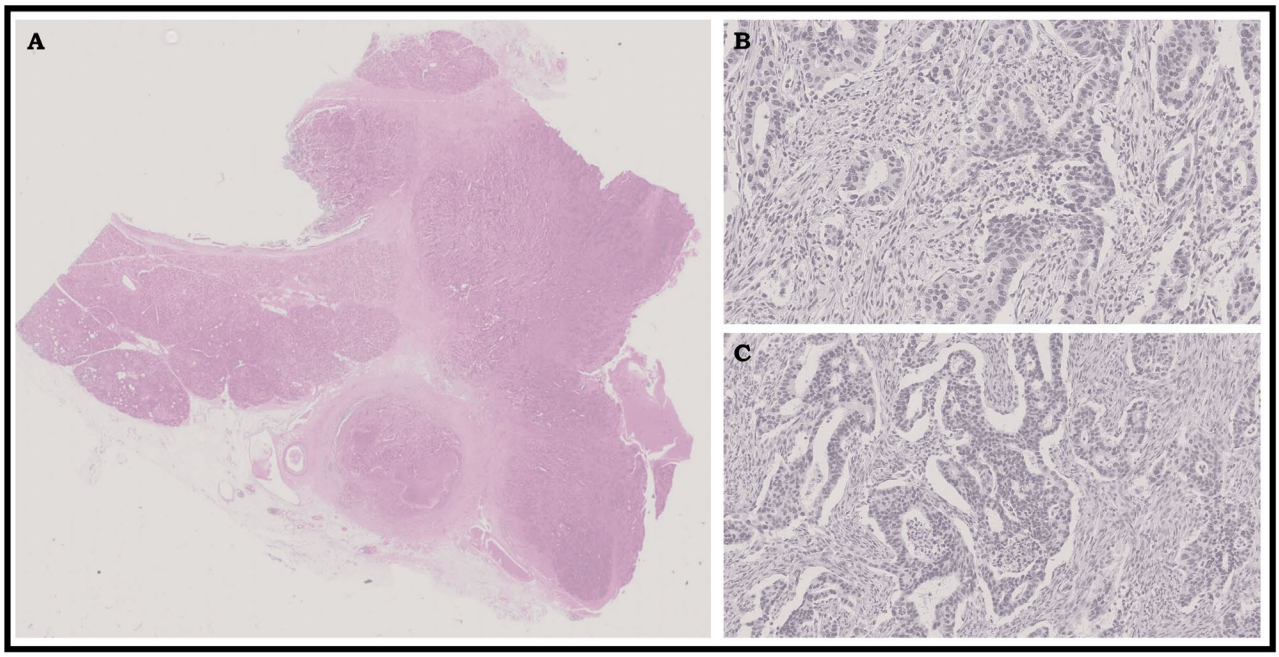

Figure 1: Case 8 – Coronal section showing ampullary adenocarcinoma (A; hematoxylin and eosin, X0,5); SMARCA4 loss pattern (B; immunohistochemistry, X25); SMARCB1 retained pattern (C; immunohistochemistry, X25).

SMARCB1 integrating analysis (protein expression and dysfunctional gene alterations) in a pilot retrospective cohort of 9 AACs, constituted by 2 mixed histological type (22.2%), 2 BP (22.2%), and 5 INT (55.6%), respectively. Immunohistochemical analysis of SMARCA4 and SMARCB1 proteins was performed following standardized procedures with primary monoclonal antibodies against SMARCA4 (BRG1, 1:50; Abcam), and SMARCB1 (clone A-5 mouse, 1:100; Santa Cruz), on the automated platform Autostainer Link 48 (Dako, Carpinteria, CA, USA). After microdissection and extraction of neoplastic cellularity from unstained slides, DNA was sequencing with a comprehensive commercially available NGS panel (Oncomine™ Tumor Mutation Load Assay, Thermo Fisher Scientifics) that allows the simultaneous analysis of single nucleotide variants, copy number variations, and insertions/deletions in 409 cancer related genes (including *SMARCA4* and *SMARCB1*) on NGS platform.

Immunohistochemistry showed a retained pattern (nuclear staining in neoplastic cells) in a single case (11%) and in 6 cases (66%) for SMARCA4 SMARCB1, respectively. A loss pattern (absence of nuclear immunostaining in neoplastic cells with preserved staining in non-neoplastic tissue) was identified in 8 cases (89%) for SMARCA4 (Fig. 1). In 3 cases, SMARCB1 evaluation was not reliable due to the absence of immunostaining in internal controls.

SMARCA4 gene alterations were detected in 77.7% (7 out of 9) of cases but removing a median of 10.1 (ranging from 1.0 to 20.0) synonymous mutations, potentially impacting *SMARCA4* alterations were identified. Moreover, all *SMARCA4* hotspot alterations were detected in bromodomain (*BRK*) domain (Tab. I). *In vivo* studies demonstrated that the *BRK* domain is crucial for interacting acetylated histone H3K14 suggesting a promising target for selective inhibition [5]. Similarly, *SMARCB1* molecular alterations were observed in 5 out of 9 (55.6%) cases (Tab. I). Among them, a median of 1.9 (ranging from 1.0 to 8.0) non-synonymous mutations was detected. In addition, concomitant molecular alterations in *SMARCA4* and *SMARCB1* were also identified in 5 out of 9 (55.6%) AAC patients. This finding has been described showing a *SMARCA4* and *SMARCB1* simultaneous loss pattern in undifferentiated endometrial carcinoma [2] and concomitant *SMARCB1* alteration in 2/56 malignant rhabdoid tumor [6] and in 3/19 ossifying fibromyxoid tumor [7]. The higher frequency rate of *SMARCA4*/*SMARCB1* concomitant molecular alterations in our series of AAC patients can suggests their eligibility in clinical trials investigating *SMARCA4* deficient tu-

Table I. SMARCA4 and SMARCB1 gene mutations grouped for functional protein effect.

ID	Mutations in SMARCA4*	Mutations in SMARCB1*	Non-sense mutations		Missense Mutations		Clinically significant mutations
			SMARCA4	SMARCB1	SMARCA4	SMARCB1	
SMA1	4	0	0	0	1	0	N.O.
SMA2	47	16	1	2	18	5	SMARCA4 p.Arg905Cys (c.2713C > T) MAF: 9.4% - Type: VUS
SMA3	40	4	2	1	16	0	SMARCA4 p.Ala1002Val (c.3005C > T) MAF: 10.5% - Type: VUS
SMA4	14	6	0	1	5	0	SMARCB1 p.Trp51Ter (c.152G > A) MAF: 55.9% - Type: Pathogenic
SMA5	27	2	0	0	10	0	SMARCA4 p.Gly1644Ser (c.4930G > A) MAF: 16.0% - Type: VUS
SMA6	0	0	-	-	-	-	N.O.
SMA7	23	8	2	0	8	2	N.O.
SMA8	2	0	0	0	1	0	N.O.
SMA9	0	0	-	-	-	-	N.O.

MAF (Mutant Allelic Frequence); N.O. (Not Observed); VUS (Variant Uncertain Significance).

* Mutations count includes all synonymous, non-sense, missense and unknown mutations. Clinical significance of reported mutations refers to ClinVar database (last update at submission date).

mor patients administered with Immune Check Point Inhibitors (ICIs) [8]. Moreover, in accordance with ClinVar public database, variant of uncertain significance (VUS) in 3 (33.3%) patients was found whereas a likely pathogenic alteration was detected in a single instance. In addition, a *SMARCB1* pathogenic alteration was identified in a single patient (Tab. I).

Two patients (SMA#6, #9) did not meet quality metrics (average reads per amplicon < 100) from NGS analysis and showed a *SMARCA4*/*SMARCB1* wild type molecular profile. In this regard, pre-analytical management of tissue sample represents a crucial issue strongly related to the successful rate of molecular analysis. In our series, three patients (SMA#2, #3, #5) (33.4%) showed *TP53* molecular alterations. In addition, *KRAS* non-synonymous variants were detected in 4 out of 9 (44.4%) patients (SMA#1, #2, #3, #7). Moreover, *APC* and *ERBB3* molecular alterations were also found in accordance with literature data, while *CDKN2A* variants were not detected.

SMARCA4 immunostaining matched with the *SMARCA4* molecular profile in 66.0% of cases. In one case, *SMARCA4* missense mutation showed a preserved nuclear immunostaining. SMARCB1 immunostaining matched with *SMARCB1* genic status in 22.0% of cases. Beyond the 33.0% of cases not evaluable for immunostaining, a retained protein immunostaining (45.0%) was simultaneous inspected with *SMARCB1* molecular alterations. This data can suggest investigating *SMARCA4* and *SMARCB1* in AAC by molecular assays. Of note, concomitant *SMARCA4-SMARCA2* inactivating mutations were also identified in thoracic neoplasms, suggesting an evolutionary double hit model in sarcomatoid/undifferentiated tumors [9]. Similarly, simultaneous *SMARCA-SMARCB* inactivating mutations play a synergic effect on dysfunctional chromatin remodeling supporting the hypothesis on the pivotal role of SMARC complex in regulating cell cycle [10].

Our study is affected by several limitations. The first is small series of AAC patients that requires further investigation to confirm molecular records; the second one is that functional studies and methylation-based assays should be used to validate molecular alterations detected in promising biomarkers by wide NGS panel. Finally, clinical information on survival and therapy is lacking. Further data to validate the evolutionary molecular landscape of *SMARC* deficient AAC patients to implement these findings in the clinical practice are needed.

In conclusion, *SMARC* investigation in AAC can suggest a peculiar molecular pathway, suitable for possible clinical management and targeted therapy in a subset of patients. Methods for *SMARC* protein and genic alteration assessment, by both immunohistochemistry and molecular assays need further validation with a pre-analytic standardization.

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Conflict of interest statement

P.P. received personal fees (as consultant and/or speaker bureau) unrelated to the current work from MSD, Astellas, Pierre Fabre, GSK, Diatech Pharmacogenetics, Incyte, Bristol. F.P. received personal fees (as consultant and/or speaker bureau) unrelated to the current work from Menarini. Roche and Agilent U.M. received personal fees (as consultant and/or speaker bureau) unrelated to the current work from Boehringer Ingelheim, Roche, MSD, Amgen, Thermo Fisher Scientifics, Eli Lilly, Diaceutics, GSK, Merck and AstraZeneca, Janssen, Diatech, Novartis and Hedera. G.T. reports personal fees (as speaker bureau or advisor) unrelated to the current work from Roche, MSD, Pfizer, Boehringer Ingelheim, Eli Lilly, BMS, GSK, Menarini, AstraZeneca, Amgen and Bayer. P.G. has received personal fees (as consultant and/or speaker bureau) unrelated to the current work from Boehringer Ingelheim, Roche, MSD, Amgen, Eli Lilly, AstraZeneca, Pfizer and Novartis.

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Authors contribution

Conceptualization: PP, FP, PG and UM. Methodology: all authors. Software: all authors. Validation: all authors. Formal analysis: all authors. Investigation: all authors. Resources: all authors. Data curation: all authors. Writing – original draft preparation: PP, FP. Writing – review and editing: all authors. Visualization: all authors. Supervision: PG, UM. Project administration: PG, UM. Funding acquisition: PP.

Ethical consideration

All information regarding human material was managed using anonymous numerical codes, and all samples were handled in compliance with the Helsinki Declaration.

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