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Porocarcinomas with *PAK1/2/3* fusions: a series of 12 cases

Aims: Porocarcinoma is a malignant sweat gland tumour differentiated toward the upper part of the sweat duct and may arise from the transformation of a preexisting benign poroma. In 2019, Sekine *et al.* demonstrated the presence of *YAP1::MAML2* and *YAP1::NUTM1* fusions in most poromas and porocarcinomas. Recently, our group identified *PAK2*-fusions in a subset of benign poromas. Herein we report a series of 12 porocarcinoma cases harbouring *PAK1/2/3* fusions.

Methods and Results: Five patients were male and the median age was 79 years (ranges: 59–95). Tumours were located on the trunk (n = 7), on the thigh (n = 3), neck (n = 1), or groin area (n = 1).

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Maxime Battistella and Arnaud de la Fouchardière equally contributed to the present study.

Abbreviations: MAML2, mastermind like transcriptional coactivator 2; NUT, NUT midline carcinoma family member 1; PAK, p21 (RAC1) activated kinase; YAP1, Yes1 associated transcriptional regulator.

Four patients developed distant metastases. Microscopically, seven cases harboured a benign poroma component and a malignant invasive part. Ductal formations were observed in all, while infundibular/horn cysts and cells with vacuolated cytoplasm were detected in seven and six tumours, respectively. In three cases, the invasive component consisted of a proliferation of elongated cells, some of which formed pseudovascular spaces, whereas the others harboured a predominant solid or trabecular growth pattern. Immunohistochemical staining for CEA and EMA confirmed the presence of ducts. Focal androgen receptor expression was detected in three specimens. Whole RNA sequencing evidenced *LAMTOR1::PAK1* (n = 2), *ZDHHC5::PAK1* (n = 2), *DLG1::PAK2*, *CTDSP1::PAK1*, *CTNND1::PAK1*, *SSR1::PAK3*, *CTNNA1::PAK2*, *RNF13::PAK2*, *ROBO1::PAK2*, and *CD47::PAK2*. Activating mutation of *HRAS* (G13V, n = 3, G13R, n = 1, Q61L, n = 2) was present in six cases.
Conclusion: Our study suggests that *PAK1/2/3* fusions is the oncogenic driver of a subset of porocarcinomas lacking *YAP1* rearrangement.

Introduction

Poromas are benign tumours differentiated toward the upper portion of the sweat gland apparatus.[1–3] Four histologic variants, namely, hidroacanthoma simplex, poroma, poroid hidradenoma, and dermal duct tumour are described.[1–3] Poroma can either be eccrine or apocrine and some of them harbour areas of follicular and sebaceous differentiation.[4,5] Porocarcinoma constitutes the malignant counterpart of poroma and arises *de novo* or from a preexisting poroma.[6] Prognosis of porocarcinoma is poorly defined,[7] and no uniform treatment guideline is currently available at the metastatic stage.[8]

The identification of recurrent oncogenic drivers in cutaneous adnexal tumours largely contributed to improve the classification of these neoplasms and might in addition allow the identification of new therapeutic targets for metastatic malignant cases.[9–11] In 2019, Sekine *et al.* demonstrated recurrent *YAP1::MAML2* and *YAP1::NUTM1* in most poromas and porocarcinomas,[12] *YAP1::NUTM1* rearrangements being more prevalent in the latter setting. The high frequency of such alterations,[12,13] together with demonstration of their oncogenic properties in preclinical models,[14] strongly suggest that *YAP1* rearrangements are the oncogenic driver of the majority of poromas and porocarcinomas.

Recently, our group identified recurrent *PAK2* (*p21* (*RAC1*) *activated kinase 2*)-fusions in a subset of benign poroma cases harbouring follicular and sebaceous differentiation.[15] Importantly, such *PAK2* fusions were not observed in conventional eccrine or apocrine poromas and were mutually exclusive with *YAP1*-rearrangements.[15] Moreover, fusions involving *PAK2* or the closely related *PAK1* gene have been previously reported in two metastatic porocarcinoma cases (n = 1, respectively) lacking *YAP1* fusion,[11,16] although it was unclear whether the fusion represented the initiating driver event in such cases, as only metastatic tumour was analysed.

Herein, we report the clinical, morphologic, and immunohistochemical and genetic features of a series of 12 *PAK1/2/3*-rearranged porocarcinoma cases.

Methods

CASE SELECTION

Following the identification of one index case with *PAK1* fusion, porocarcinoma cases with similar morphology and lacking *YAP1*-rearrangment were identified from the consultation's files of the authors (F.D., T.K., A.F., D.W., D.K., M.B.). Only primary tumours were considered for inclusion. To note, histologic and molecular features of the metastases were previously published[11] for Case #5,; however, this case was still included in the present study in order to provide a description of the primary tumour. Moreover, microscopic features of Case #12 have been previously reported without molecular analysis.[5] The design of this retrospective study was in agreement with the requirements for the use of biological material in research proposed by our institutional ethics guidelines (Local Ethics Committee in Human Research, Tours, France; no. ID RCB2009-A01056-51). The diagnosis of porocarcinoma was confirmed by two pathologists (M.B., T.K.) according to the criteria proposed by the WHO classification of skin tumours,[17] i.e. skin tumours composed of poroid cells, harbouring duct formation and signs of malignancy.

MORPHOLOGICAL ANALYSIS

The following morphologic criteria were evaluated by two pathologists (M.L., T.K.): architecture (solid,

nodular, cystic, pseudovascular, fascicular, trabecular), cytology (poroid, squamoid, spindle, epithelioid, atypia, clear cell changes), poroid differentiation (duct/gland formation, eosinophilic cuticle, decapitation secretion), follicular differentiation (infundibular/horn cyst, squamous eddies, keratin calcification), sebaceous differentiation (cells with vacuolated cytoplasm classified as focal [rare and isolated cell] or as clusters of sebocytes, others [hyalinized stroma, myxoid stroma, melanin aggregates, necrosis, mitotic count, vascular invasion]).

IMMUNOHISTOCHEMISTRY

Immunohistochemical staining for EMA, BerEP4, betacatenin, androgen receptor, cytokeratin 7, CEA, P16, P53, P63, PTEN, SOX10, YAP1 (C-Ter), NUT was performed using a Ventana BenchMark XT Platform (Roche Diagnostics, Basel, Switzerland) as instructed. Antibodies and dilutions are provided in the Supplementary Material.

WHOLE RNA SEQUENCING

Total RNA was extracted from FFPE tissue sections. Whole RNA sequencing was performed using two distinct procedures. For Cases #1, 2, 4–7, RNA sequencing was performed as previously described.[18,19] Briefly, for each sample 100 ng of total RNA was used to prepare the library with TruSeq RNA Access Library Prep Kit (Illumina, San Diego, CA, USA). Fourteen libraries were pooled at 4 nM with PhiX Control in a low-concentration spike-in (1%). Sequencing was performed (75 cycles paired-end) with NextSeq 500/550 High Output V2 Kit in NextSeq 500 (Illumina). The samples were analysed on BaseSpace sequence Hub (Illumina) with RNA-Sequencing Alignment application. The alignments were realized with Star[20] and TopHat2[21] on the GRCh37 reference genome. The fusion transcript was called STAR-Fusion,[22] FusionMap,[23] EricScript,[24] FusionCatcher,[25] and Arriba.[26] To analyse single nucleotide variants, we followed RNAseq short variant discovery best practices (proposed by the Genome analysis toolkit[27] prior functional annotation using the ANNOVAR tool).[28]

For Cases #3, 8–12, libraries were performed using the RNASseq SureSelect XTHS2 kit (Agilent, Santa Clara, CA, USA). Sequencing (101 cycles paired-end) was performed on a NextSeq 2000 platform (Illumina). Sequence alignment was done on Human genome GRCh38 and fusion calling was achieved with Star_arriba,[26] Star_fusion,[22] and Fusioncatcher.[25] Single nucleotide variant targeted detection in all genes with pathogenic mutation detected in Cases #1, 2, 4–7 (i.e. *HRAS*, *APC*, *PTEN*, *MEN1*, *PIK3CA*, and *TP53*) was performed by BAM file visualization on Alamut Visual Plus (Sophia Genetics, Switzerland).

Ward's unsupervised clustering and Uniform Manifold Approximation and Projection (UMAP) were performed in the R (v. 4.2. 2, Vienna, Austria) environment using the cluster and UMAP packages, respectively, on six PAK-rearranged porocarcinoma cases in comparison to *PAK*-fused poroma (n = 10), *YAP1*-rearranged porocarcinoma (n = 3), apocrine mixed tumour (n = 21), eccrine mixed tumour (n = 8), basal cell carcinoma (n = 8), trichogerminoma (n = 18), and Merkel cell carcinoma (n = 12). To note, a part of this control group has been previously described.[29]

Results

Twelve cases of porocarcinomas with *PAK1*, *PAK2*, or *PAK3* rearrangement were included in the present study. All characteristics of the cases are provided in Table 1 and Figures 1–5. Briefly, five patients were male and seven were women. The mean age was 79 years (range: 59–95). Tumours were located on the trunk (n = 7), on the thigh (n = 3), on the neck (n = 1), or on the groin area (n = 1). Mean size was 14 mm (range: 5–110). A multinodular appearance was noted by the physician in three cases. Four patient developed distant metastases during follow-up (median duration: 5 months, range: 1–36). Case #2 developed regional lymph nodes and lung metastases and was still alive after a 12-month follow-up. Case #5 had regional (lymph node and in-transit metastases) and distant metastatic spreading (bone, muscle, and brain metastases), finally resulting in patient death, as previously reported.[11] Lymph node metastases were identified at the diagnosis time for Cases #8 and #9 together with bone metastases in Case #9. Both patients were still alive 4 and 5 months, respectively, after the diagnosis.

Microscopic examination of the primary tumours revealed in seven cases a benign poroma component and a malignant invasive part with transition areas between the two tumour parts (Figures 1 and 2). The poroma component was composed of uniform, nonatypical poroid cells with scant cytoplasm and small, round nuclei. Duct formations were observed in all cases. Horn cysts (n = 7), clear cell changes (n = 6), and melanin deposit (n = 1) were also evidenced in some cases. Cells harbouring abundant, vacuolated

Table 1. Clinical, microscopic, and genetic features of the cases

Clinical features	Case #1	Case #2	Case #3	Case #4	Case #5	Case #6	Case #7	Case #8	Case #9	Case #10	Case #11	Case #12
Age (years)	80	80	75	82	59	93	95	77	60	75	63	89
Sex	M	F	M	M	M	F	F	F	F	F	M	F
Location	Abdomen	Thigh	Back	Chest wall	Right Scapula	Thigh	Breast	Buttock	Neck	Groin area	Thigh	Buttock
Tumour size (mm)	26	110	11	46	8	19	30	15	9	5	5	12
Follow up (months)	3	12	16	NA	36	–	1	4	5	2	NA	18
Follow up (event)	–	Lymph node and lung metastases	–	NA	Skin, lymph node, muscle, bone and brain metastases	NA	–	Bone and lymph node metastases	Regional lymph node metastases	–	NA	–

	Case #1		Case #2	Case #3		Case #4		Case #5	Case #6		Case #7		Case #8		Case #9	Case #10	Case #11	Case #12	
Morphologic features	Poroma	Carcinoma	Carcinoma	Poroma	Carcinoma	Poroma	Poroma	Carcinoma	Poroma	Carcinoma	Poroma	Carcinoma	Poroma	Carcinoma	Carcinoma	Carcinoma	Carcinoma	Poroma	Carcinoma
Epidermis																			
Connection	Multiple	+	Multiple	–	+	Multiple	Multiple	Multiple	In situ	Multiple	Multiple	Multiple	Multiple	Multiple	–	Multiple	Multiples	Multipes	+
Ulceration	–	+	+	+	+	–	–	+	–	–	–	+	+	+	–	–	–	–	+
Location																			
Dermis	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Subcutaneous	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–
Growth pattern	Expansive	Infiltrative	Expansive	Expansive	Infiltrative	Expansive	Infiltrative	Infiltrative	NA	Infiltrative	Expansive	Infiltrative	Expansive	Infiltrative	Infiltrative	Expansive	Expansive	Expansive	Infiltrative
Architecture																			
Solid	+	–	+	+	–	+	+	+	–	+	+	+	+	–	+	+	–	+	–
Nodular	–	–	Focal	–	–	–	+	–		–	–	–	–	–	+	+	–	+	–
Cystic	+	–	–	–	–	+	–	–		–	–	–	–	–	–	–	+	–	–
Pseudovascular	–	+	–	–	+	–	–	–		–	–	–	–	–	–	–	–	–	+
Fascicular	–	+	–	–	–	–	–	–		–	–	–	–	+	–	–	–	–	–
Trabecular	–	–	–	–	–	–	–	+		+	–	+	+	+	–	–	+	+	–
Cytology																			
Poroid	+	–	++	+	–	++	+	+	++	++	+	+	++	–	+	+	+	+	–
Squamoid	+	–	+	–	–	+	++	–	–	–	–	–	–	+	–	–	+	–	–
Spindle	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Epithelioid	–	–	–	–	+	–	–	+	–	+	–	–	–	+	–	+	+	–	–
Atypia	–	Moderate	Moderate	–	Moderate	–	Moderate	Moderate	–	Moderate	–	Moderate	–	High	Moderate	Moderate	High	–	Moderate
Clear cell changes	+	–	+	–	–	+	–	–	+	–	+	–	+	–	–	–	–	–	–

Table 1. (*Continued*)

Morphologic features	Case #1 Poroma	Case #1 Carcinoma	Case #2 Carcinoma	Case #3 Poroma	Case #3 Carcinoma	Case #4 Poroma	Case #4 Poroma	Case #5 Carcinoma	Case #6 Poroma	Case #6 Carcinoma	Case #7 Poroma	Case #7 Carcinoma	Case #8 Poroma	Case #8 Carcinoma	Case #9 Carcinoma	Case #10 Carcinoma	Case #11 Carcinoma	Case #12 Poroma	Case #12 Carcinoma
Poroid differentiation																			
Duct/gland formation	+	+	+	+	+	+	–	+ focal	+	+	+	+	+	+	+	+	+	+	–
Eosinophilic cuticle	–	–	+	+	–	++	–	–	–	–	–	–	+	–	–	–	+	+	–
Decapitation secretion	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Follicular differentiation																			
Infundibular/Horn cyst	+	–	+	+	–	++	–	–	Focal	–	+	–	–	–	–	–	+	–	–
Squamous eddies	–	–	+	+	–	+	–	–	–	–	–	–	+	–	+	–	–	–	–
Keratin calcification	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sebaceous differentiation																			
Cells with vacuolated cytoplasm	Focal	–	Clusters	–	–	Clusters	–	–	Focal	Focal	Focal	–	–	–	–	–	–	Clusters	–
Others																			
Hyalinized stroma	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
Myxoid stroma	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	+
Melanin aggregates	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Necrosis	–	–	+	–	–	–	+	–	–	+	–	–	–	–	+	+	–	–	–
Mitotic count/ mm^2	–	8	6	–	8	3	5	5	2	9	1	2	1	7	5	4	6	–	3
Vascular invasion	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

IHC features	Case #1	Case #2	Case #3	Case #4	Case #5	Case #6	Case #7	Case #8	Case #9	Case #10	Case #11	Case #12
EMA	Sebocytes + ducts	Sebocytes + ducts	Ducts	Heterogenous	Focal	Heterogenous	Heterogenous	NA	NA	NA	NA	NA
BerEP4	—	Heterogenous	Ducts	Ducts	—	—	—	NA	Heterogeneous	—	-	NA
Androgen Rec	—	<10%	—	<10%	—	—	—	—	<5%	—	-	NA
CK7	Ducts	Diffuse	Heterogenous	Focal	—	Ducts	Ducts	NA	NA	NA	NA	NA
CEA	Ducts	Ducts	Ducts	Ducts	Ducts	Ducts	Ducts	Ducts	Ducts	Ducts	Ducts	NA
P63	Diffuse	Diffuse	Diffuse	Diffuse	Diffuse	Heterogenous	Diffuse	NA	Diffuse	Diffuse	Diffuse	NA
SOX10	—	—	— (Mel.Hyp)	— (Mel.Hyp)	—	—	—	NA	—	—	-	NA

Table 1. (*Continued*)

IHC features	Case #1	Case #2	Case #3	Case #4	Case #5	Case #6	Case #7	Case #8	Case #9	Case #10	Case #11	Case #12
YAP1 (C-Ter)	Preserved	Preserved	Preserved	Preserved	Preserved	Preserved	Preserved	Preserved	Preserved	Preserved	Preserved	NA
NUT	—	—	—	—	—	—	—	—	—	—	-	NA
Genetic features	Case #1	Case #2	Case #3	Case #4	Case #5	Case #6	Case #7	Case #8	Case #9	Case #10	Case #11	Case #12
Fusion transcripts	*LAMTOR1::PAK1*	*ZDHHC5::PAK1*	*DLG1::PAK2*	*CTDSP1::PAK1*	*CTNND1::PAK1*	*SSR1::PAK3*	*LAMTOR1::PAK1*	*CTNNA1::PAK2*	*RNF13::PAK2*	*ROBO1::PAK2*	*ZDHHC5::PAK1*	*CD47::PAK2*
HRAS mutations	*HRAS* p.G13V	*HRAS* p.Q61L	*HRAS* p.G13V	–	–	–	–	*HRAS* p.G13R	–	*HRAS* p.G13V	–	*HRAS* p.Q61L
APC	—	*APC* p.R1146H *APC* p.T2813I	–	–	*APC* p.Q1928*	–	–	–	–	–	*APC* p.K716*	*APC* p.Q793*
PTEN	–	–	–	–	–	*PTEN* p.V216Cfs*27	–	–	–	–	–	–
MEN1	*MEN1* p.Q166*			–	–		–	*MEN1* p.K562*	–	–	–	–
PIK3CA mutations	–	–	–	*PIK3CA* p.E542K	–	–	–	–	–	–	–	–
TP53	–	–	–	–	*TP53* p.G245D	*TP53* p.R196*	*TP53* p.R273H	*TP53* p.R248Q	–	–	–	–

"–", absence; "+", presence; "++", predominant; Focal, presence of sparse isolated cells with vacuolated cytoplasm; Mel Hyp, melanocytes hyperplasia; NA, not available.

cytoplasm and scalloped nuclear membrane suggesting a sebocytic differentiation ($n = 6$, either isolated cells ($n = 3$) or forming clusters ($n = 3$)) were present in the benign ($n = 5$) and/or in the malignant part ($n = 2$) of the tumours. Five cases were devoid of both sebaceous differentiation and infundibulocystic structures.

In three specimens, the invasive component consisted of a proliferation of elongated cells, some of which formed ducts or were organized as pseudovascular spaces (Cases #1, #3, and #12). In the others, the invasive component had a predominant solid or trabecular growth pattern with cohesive tumour cells harbouring abundant cytoplasm. Cytologic atypia was moderate in 10 cases and high in two cases. Numerous mitotic figures were observed in all cases (median count in the malignant component: six mitotic figures/mm^2, range: 2–9). Area of necrosis "en masse" was observed in five cases.

Immunohistochemical staining (Figure 3) for CEA and EMA confirmed the presence of ducts in all cases. Focal androgen receptor expression was detected in the malignant component (in poroid cells) in three cases. Diffuse positivity of p40 and lack of SOX10 expression was also demonstrated. Moreover, by contrast to *YAP1*-rearranged porocarcinoma, YAP1 (C-terminal part) expression was preserved and no NUT positivity was detected.

Whole RNA sequencing evidenced *LAMTOR1::PAK1* ($n = 2$), *ZDHHC5::PAK1* ($n = 2$), *DLG1::PAK2*, *CTDSP1::PAK1*, *CTNND1::PAK1*, *SSR1::PAK3*, *CTNNA1::PAK2*, *RNF13::PAK2*, *ROBO1::PAK2*, and *CD47::PAK2* (Figure 4). Importantly, as previously published *PAK2*-fusions in poromas,[15] the *PAK1/2/3* rearrangements detected in the present study result in loss of the N-terminal part of the protein, including the regulatory domain, while the full in-frame kinase domain was always preserved.

Single nucleotide variants calling further demonstrated in association to the *PAK* fusion the presence of recurrent pathogenic mutations in *HRAS* (G13V, $n = 3$, G13R, $n = 1$, Q61L, $n = 2$), *TP53* ($n = 4$), *APC* ($n = 4$), and *MEN1* ($n = 2$). To further confirm these results, expression of p53, beta-catenin, a downstream target of APC, were then evaluated by immunohistochemistry in five porocarcinoma cases with material available (Cases #1, 2, 4, 6, and 7). Moreover, since *CDKN2A* and *PTEN* inactivation has been reported in porocarcinoma,[30] including cases with *PAK1/2* fusions,[11,16] p16 and PTEN expression was also evaluated (Table S1 and Figure S1). Such analysis demonstrated abnormal P53 expression (either loss or overexpression) in the malignant

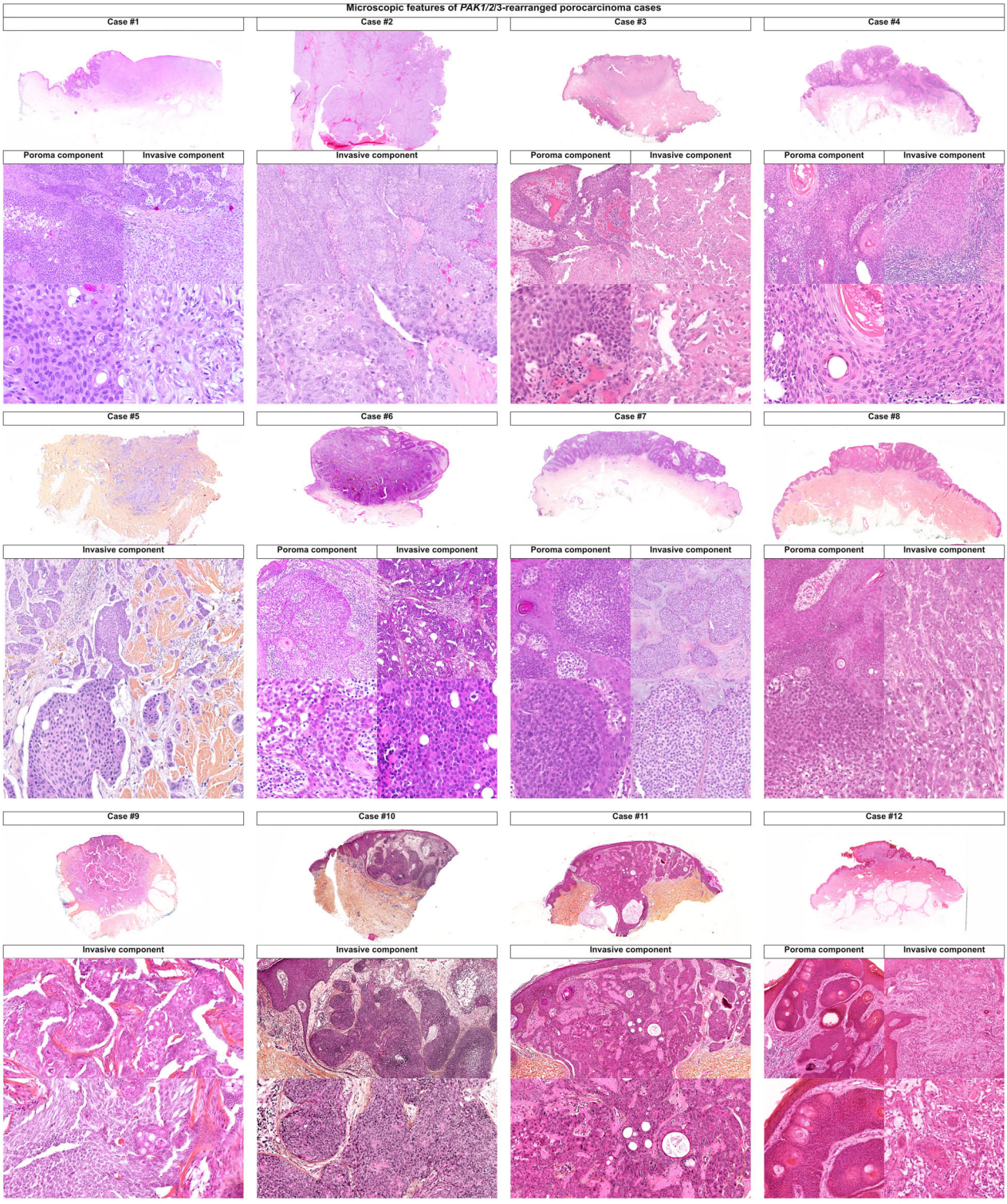

Figure 1. Morphologic features of the *PAK1/2/3*-rearranged porocarcinoma cases included in this study. Seven cases harboured a benign poroma component in association to the invasive part (Cases #1, #3, #4, #6–8, and #12). The poroma component was composed of uniform, nonatypical poroid cells. Ductal formations, follicular cysts, and sparse sebocytes were present in most cases. The invasive component consisted of a proliferation of elongated cells sometimes forming ducts or organized as pseudovascular spaces (Cases #1, #3, and #12) or harboured a predominant solid/trabecular growth pattern.

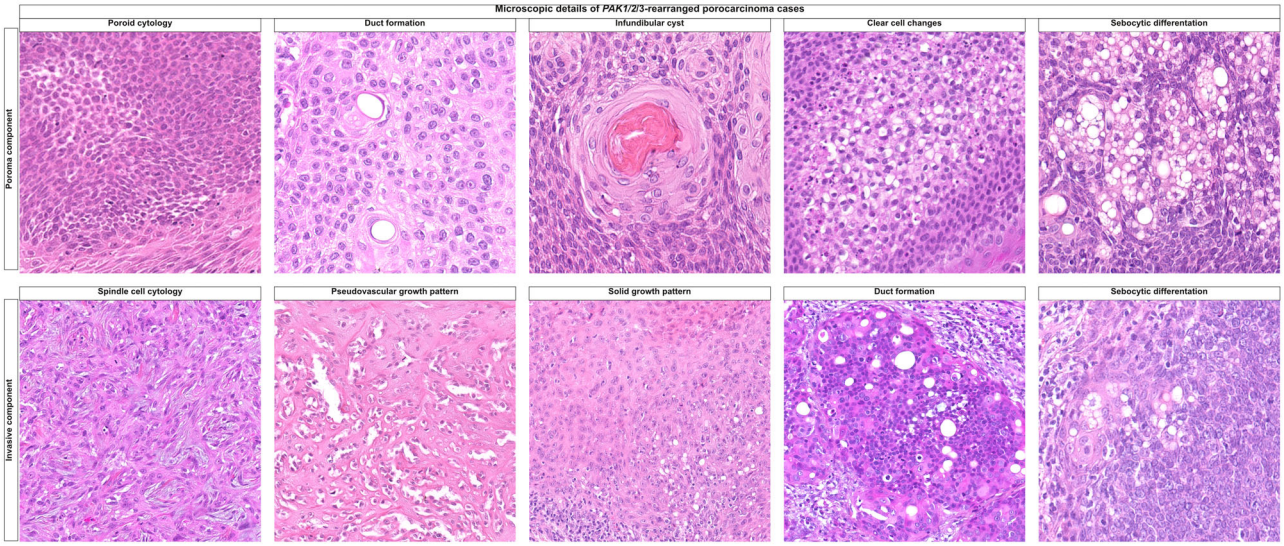

Figure 2. Microscopic details of *PAK1/2/3*-rearranged porocarcinoma cases. The benign poroma component (upper panel) was mostly composed of small nonatypical poroid cells associated with duct formation. Infundibulocystic structures and clear cells changes were frequently observed. Sebocytic differentiation may be observed in both the benign and malignant part. The malignant component (lower panel) harboured a pseudovascular or solid growth pattern.

component of all samples, while such a pattern was only observed in the benign part in one case. P16 loss was further observed in four cases restricted to the malignant part of the tumours. Loss of PTEN expression was detected in Case #6 harbouring pathogenic mutation in the *PTEN* gene. Despite recurrent *APC* mutations observed in our cohort, no nuclear accumulation of the beta-catenin was detected in any specimen, including one case with *APC* mutations (Case #2).

Clustering analyses using gene expression profiling were performed on six *PAK*-fused porocarcinoma tumours (Cases #1, 2, 4–7) and 80 other adnexal tumours used as controls, including *PAK*-fused poroma (n = 10), *YAP1*-rearranged porocarcinoma (n = 3), apocrine cutaneous mixed tumour (n = 21), eccrine cutaneous mixed tumour (n = 8), basal cell carcinoma (n = 8), trichogerminoma (n = 18), and Merkel cell carcinoma (n = 12). The UMAP is shown in Figure 5 and comparative analysis by genes ontology of the transcription profile of *PAK*-rearranged tumours and controls is available in Figure S2. Such analysis confirmed that, together with *PAK*-rearranged poroma and *YAP1*-rearranged porocarcinoma cases, *PAK*-rearranged porocarcinoma cases constitute a homogeneous and distinct tumour group, with a large transcriptomic distance to other neoplasms used as controls. Interestingly, as previously described,[11] high EGFR expression was observed in both *PAK*- and *YAP1*-rearranged porocarcinoma cases compared to other skin tumours (Figure S3).

Finally, to clarify whether *PAK1* fusions are restricted to malignancy in poroid tumours, we retrospectively investigated cases previously analysed by RNA sequencing (Centre Léon Bérard, Lyon, France) and identified a *FGFR2::PAK1* fusion in a benign poroma-poroid hidradenoma variant with follicular and sebaceous differentiation (Figure S4) lacking *PAK2* fusion. This specimen consisted of a 1-cm diameter nodule with no connection with the epidermis. The tumour harboured a macro- and micronodular architecture and was comprised of poroid cells with scant cytoplasm and round nuclei. Large infundibular cysts, ducts with eosinophilic cuticles, and sparse sebocytes were present. A distinctive feature was the presence of clusters of cells with clear and abundant cytoplasm within the tumour. Cytologic atypia, necrosis, or mitotic figures were absent.

Discussion

Identification of recurrent oncogenic drivers in rare cancers is crucial to improving precise systematic tumour classification, and also might provide a rationale to develop new therapeutic options.[31] In 2019, Sekine *et al.* demonstrated the presence of *YAP1::MAML2* and *YAP1::NUTM1* fusions in the majority of poromas and porocarcinomas.[12] Together with WWTR1/TAZ, YAP1 protein is a downstream effector

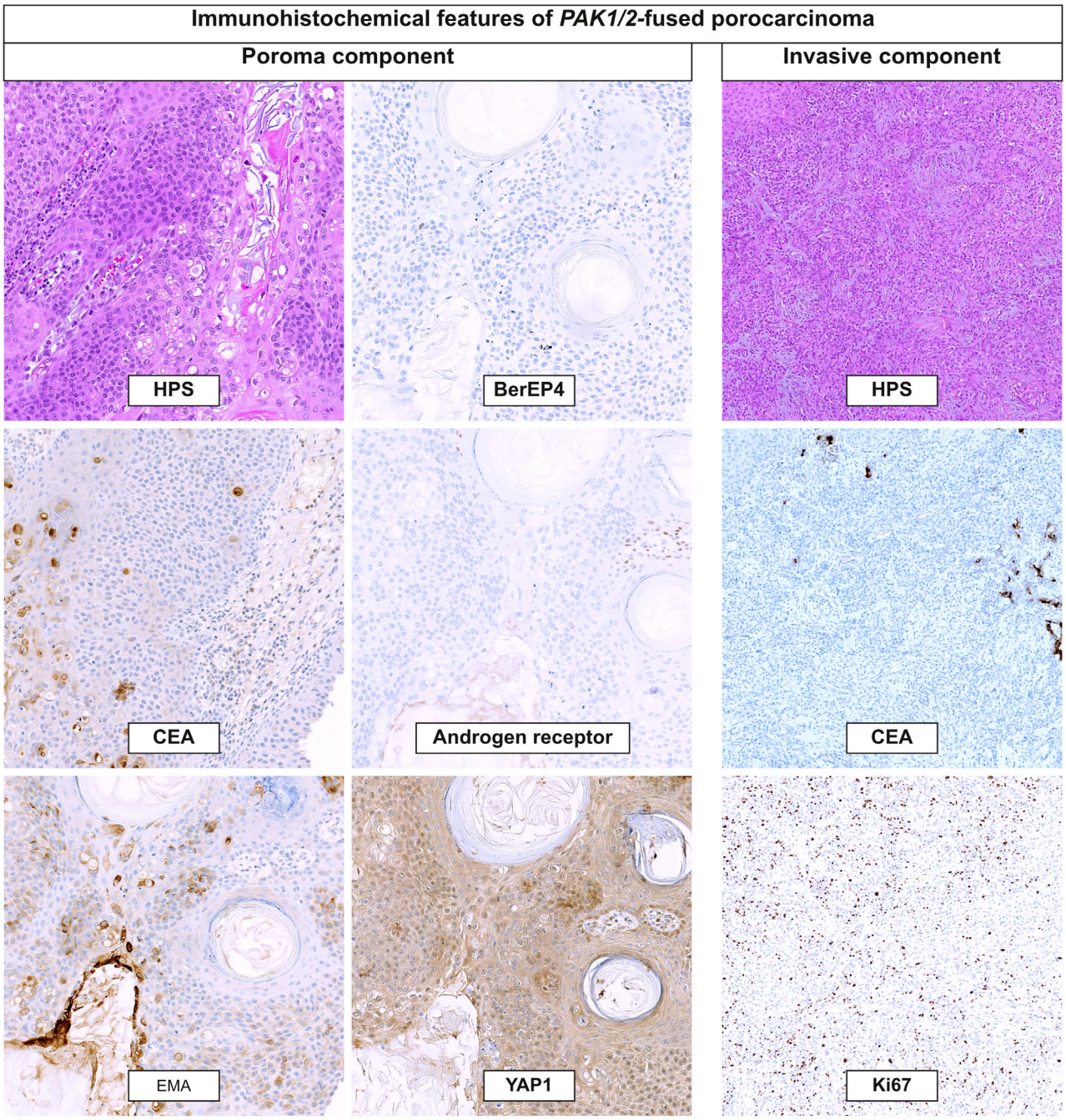

Figure 3. Immunohistochemical features of the cases. Case #1 is depicted. EMA and CEA confirmed the presence of ducts in the poroma and in the invasive part. No expression of the androgen receptor and BerEP4 was observed. Preserved expression of YAP1 reflected the lack of *YAP1* rearrangement. Finally, Ki67 confirmed high proliferation index in the invasive part.

of the Hippo pathway, a signalling pathway involved in tissue development and homeostasis.[32] Formation of a complex in the nucleus between YAP1 and the TEAD transcription factor induces the transcription of target genes and cell proliferation.[32] However, TEAD activity is downregulated by the Hippo pathway through the sequestration of TAZ and YAP1 in the cytoplasm and degradation of these proteins.[14] In tumours, rearrangement of the *YAP1* gene leads to the expression of a fusion protein constitutively located in the nucleus and insensitive to the Hippo pathway repression.[14] Oncogenic properties of such fusion proteins have been

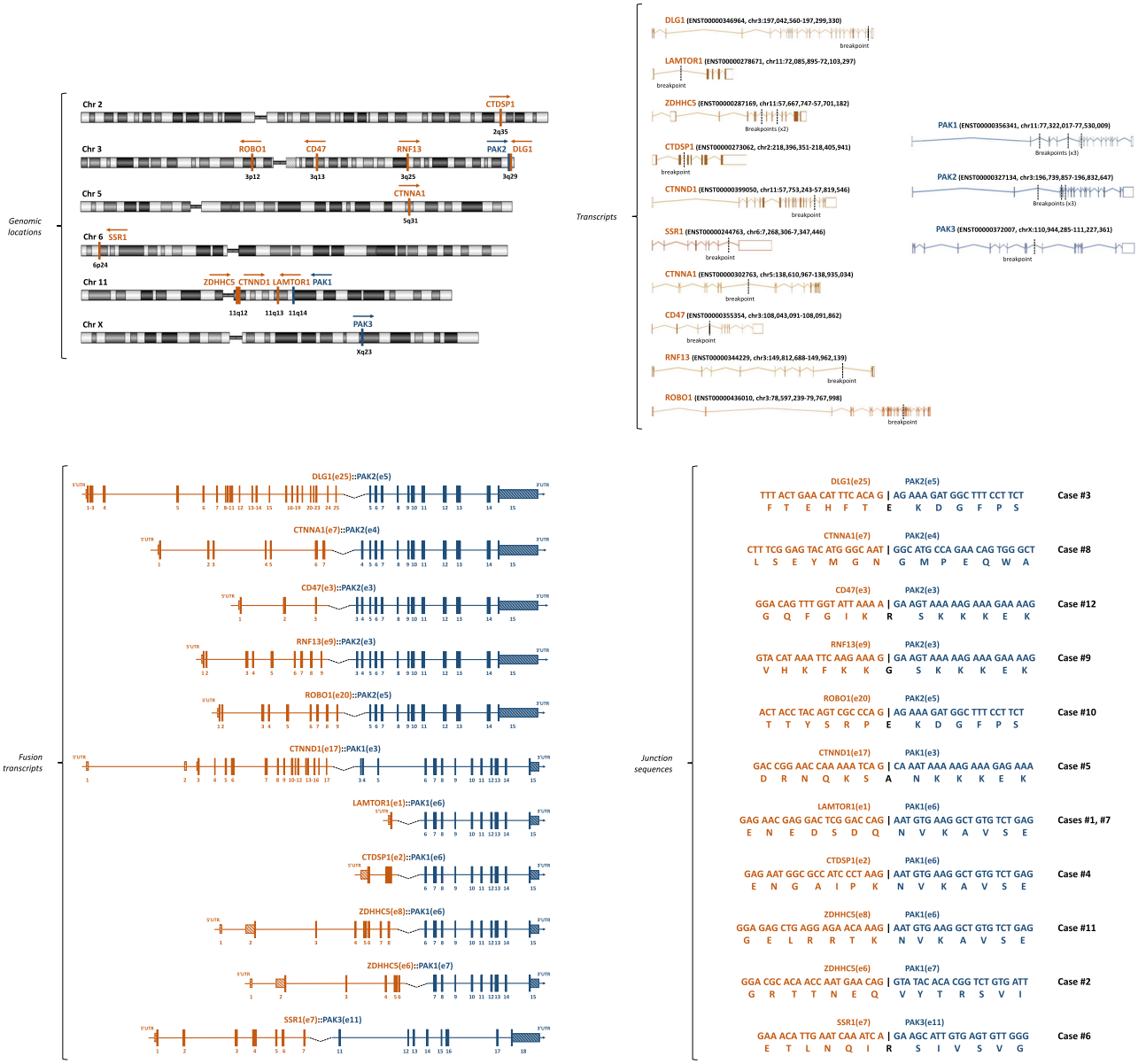

Figure 4. Schematic representation of the *PAK1/2/3* fusion transcripts. The *LAMTOR1::PAK1* ($n = 2$), *ZDHHC5::PAK1* ($n = 2$), *DLG1::PAK2*, *CTDSP1::PAK1*, *CTNND1::PAK1*, *SSR1::PAK3*, *CTNNA1::PAK2*, *RNF13::PAK2*, *ROBO1::PAK2*, and *CD47::PAK2* fusions are depicted.

demonstrated in mice models,[14] and therapeutic uses of TEAD inhibitors are currently under investigation for patients with *YAP1*-rearranged tumours in clinical trials (NCT05228015).

PAK1 and PAK2 have recently been identified as main regulators of YAP1 activity.[33,34] In the present study, we provide evidence that *PAK1/2/3* fusions constitute an oncogenic driver alternative to *YAP1*-fusion in a subset of porocarcinomas. PAKs proteins are serine–threonine kinases interacting with the Rho GTPases RAC1 and CDC42.[35,36] These proteins have been shown to be upregulated in hematologic malignancies and solid tumours and are associated with tumour growth, invasiveness, and therapeutic resistance.[35–37] As an example, PAK1 depletion in colorectal cancer led to growth tumour arrest and restored immune response.[38] Accordingly PAK inhibitors have been proposed as a potential therapeutic option in these cases[35] and might also constitute a therapeutic option for metastatic *PAK1/2/3* fused porocarcinoma.

Although more than 50 cases of *YAP1*-rearranged porocarcinoma have been reported in the literature,

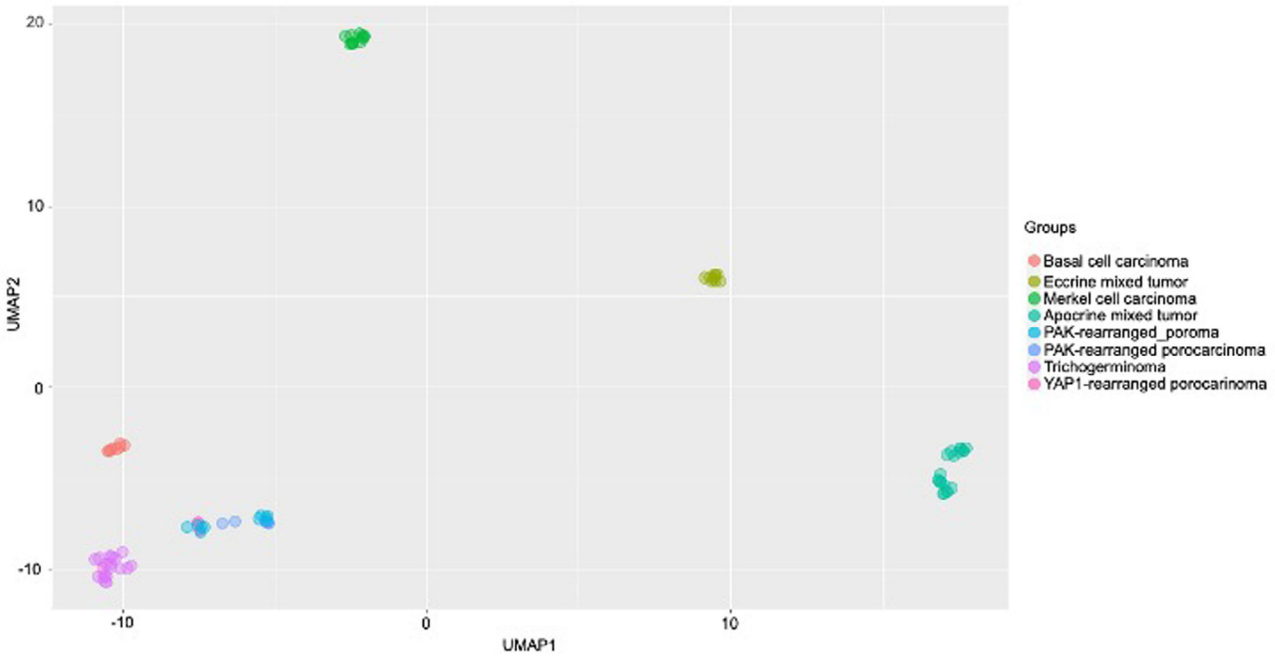

Figure 5. UMAP projection of the transcriptome of six *PAK*-fused porocarcinoma tumours (Cases #1, 2, 4–7) and 80 controls. The control group includes adnexal tumours, including *PAK*-fused poroma (n = 10), *YAP1*-rearranged porocarcinoma (n = 3), apocrine mixed tumour (n = 21), eccrine mixed tumour (n = 8), basal cell carcinoma (n = 8), trichogerminoma (n = 18), and Merkel cell carcinoma (n = 12). The cases of *PAK*-rearranged porocarcinoma cases form a robust cluster together with *PAK*-rearranged poroma and *YAP1*-rearranged porocarcinoma cases.

metastasis was only reported in two of them and was restricted to the lymph node.[39,40] Although further large studies with long-term follow-up are required for confirmation, these findings might suggest that *YAP1*-rearranged porocarcinoma have an indolent course in most of the cases. By contrast, among the 13 identified patients with *PAK1/2/3*-fused porocarcinomas, five had lymph node or visceral metastases leading to death in two cases.[11,15,16] Although such findings might be due to a referral bias and/or small sample size, it is also possible that *PAK1/2/3*-rearranged porocarcinomas constitute a more aggressive entity than *YAP1*-fused porocarcinomas.

Previously, our group evidenced recurrent *PAK2* fusions in a cohort of 13 benign poromas, with follicular and/or sebaceous differentiation.[15] In two cases of this series, an atypical component composed of poorly differentiated cells with a high nucleocytoplasmic ratio, enlarged nuclei, enhanced mitotic activity, and necrosis, already suggested a potential progression.[15] In the present study, we further confirmed such findings by the detection of a benign poroma component in association with the malignant tumour in seven specimens. These findings are in line with the view that *PAK1/2/3* fusions act as the initial oncogenic driver in this subset of poromas. Additional secondary acquired genetic alterations probably contribute to the tumour progression.[30] In this context, our study revealed an association with *PAK1/2/3* fusions, the presence of recurrent mutations in *HRAS* (G13V, n = 3, G13R, n = 1, Q61L, n = 2), and *TP53* (n = 4) mutations. Moreover, immunohistochemical staining revealed loss of p16 expression in the malignant component of four of the six samples investigated. Such findings are in line with previous detection of *HRAS*, *TP53*, and *CDKN2A* in porocarcinoma tumours[30,41] including cases with *YAP1*[12] and *PAK1/2*[11,16] fusions. However, although these alterations are likely to contribute to tumour progression, it is interesting to note that *HRAS* and *TP53* mutations might also be observed in poromas.[12,15,41]

While folliculosebaceous differentiation was observed in all *PAK2*-rearranged benign poroma cases of our previous series,[15] sebocytes and infundibulocystic structures were only detected in six and seven cases of the present study. These findings might either suggest that folliculosebaceous differentiation is lost upon progression, or that some poromas devoid of folliculosebaceous differentiation still harbour *PAK1/2/3* fusion.

Another discrepancy between our previous study[15] and the present one is that previously detected

fusions exclusively in the *PAK2* gene in benign poroma with folliculosebaceous differentiation,[15] while in the present work, both *PAK2* and *PAK1* fusions were detected, the latter being more prevalent (n = 6/12) than *PAK2* fusions (n = 5/12). Although these findings might indicate that *PAK1*-fusions are associated with malignancy, we retrospectively identified a *PAK1* fusion in a benign adnexal poroma with a distinctive morphology (Figure S4), confirming that *PAK1*-fused porocarcinoma may also derive from a benign precursor. By contrast, no benign poroma component was described in the two *PAK1/2* rearranged porocarcinomas described previously in the literature,[11,16] it should be noted that only a limited histologic description restricted to the metastasis was available for these cases. Importantly, histologic analysis of the primary tumour of one of these cases[11] in our study (Case #5) further confirmed the lack of a benign poroma component, as well as the absence of sebaceous and follicular differentiation, suggesting that *PAK1/2/3*-rearranged porocarcinoma can also arise *de novo* or without follicular and sebaceous differentiation. Interestingly, a cell line, namely, DDPoro26, was previously generated from Case #5[11] and characterization of the latter by fluorescence *in situ* hybridization (FISH) analysis in our laboratory confirmed the presence of the *PAK1* fusion in the cell line (data not shown). On the one hand, this result further confirms *PAK1* fusion as a clonal oncogenic driver shared by all tumour cells of this specimen, and on the other hand, validates the DDPoro26 cell line as an accurate model of *PAK1*-rearranged porocarcinoma, which might be useful for evaluating the effect of pharmacologic PAK inhibitors on tumour growth.

In summary, herein we reported a series of 12 porocarcinoma cases with *PAK1/2/3* fusions mostly arising from a preexisting poroma. Identification of these recurrent genetic alterations in a subset of porocarcinoma is likely to contribute to an accurate diagnosis of this entity and might also lead to the development of new targeted therapy for metastatic patients.

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Author contributions

TK, DW, DP, MB, and AF substantially contributed to he conception, design, acquisition, analysis interpretation of data as well as article drafting. ML, FT, AN, FD, JCB, NM, AS, TJ, FB, GF, BL, MM, DK, MLJ, PWH, BC, SM, and FJ substantially contributed to the acquisition of data, and revised the article. All authors approved the final version.

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Conflict of interest

The authors have no conflicts of interest to disclose.

Data availability statement

Data is available upon request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1.