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MOLECULAR PATHOLOGY

Targeted molecular profiling reveals genetic heterogeneity of poromas and porocarcinomas

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Summary

The genetic landscape of rare benign tumours and their malignant counterparts is still largely unexplored. While recent work showed that mutant *HRAS* is present in subsets of poromas and porocarcinomas, a more comprehensive genetic view on these rare adnexal neoplasms is lacking. Using high-coverage next generation sequencing, we investigated the mutational profile of 50 cancer-related genes in 12 cases (six poromas and six porocarcinomas). Non-synonymous mutations were found in two-thirds of both poromas and porocarcinomas. Hotspot *HRAS* mutations were identified in two poromas (p.G13R and p.Q61R) and one porocarcinoma (p.G13C). While in poromas only few cases showed single mutated genes, porocarcinomas showed greater genetic heterogeneity with up to six mutated genes per case. Recurrent *TP53* mutations were found in all porocarcinomas that harboured mutated genes. Non-recurrent mutations in porocarcinomas were found in several additional tumour suppressors (*RB1*, *APC*, *CDKN2A*, and *PTEN*), and genes implicated in PI3K-AKT and MAPK signalling pathways (*ABL1*, *PDGFRA*, *PIK3CA*, *HRAS*, and *RET*). UV-associated mutations were found in *TP53*, *APC*, *CDKN2A*, *PTEN*, and *RET*. In conclusion, our study confirms and extends the spectrum of genetic lesions in poromas and porocarcinomas. While poromas exhibited only few mutations, which did not involve *TP53*, the majority of porocarcinomas harboured UV-mediated mutations in *TP53* with some of these cases showing considerable genetic heterogeneity that may be clinically exploitable.

Key words: Poroma; porocarcinoma; UV signature; *HRAS*; *TP53*; *PIK3CA*.

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INTRODUCTION

Poroma is a benign skin tumour which is gender independent and primarily occurs on the lower parts of legs and arms in middle-aged adults. It is composed of well circumscribed solid sheets or nodules of small basaloid (poroid) cells and larger eosinophilic cuticular cells, with focal presence of small ducts lined by an eosinophilic cuticle. Three histological subtypes have been described, which largely relate to the involvement of epidermis and/or dermis: hidroacanthoma simplex, dermal duct tumour, and classic poroma.[1] Unlike poroma, porocarcinoma is a rare malignant skin tumour of the elderly (incidence is ~0.004%), mostly arising on the extremities and head.[2,3] Metastatic spread occurs in ~25% of these patients, usually via lymph nodes, and mortality in such cases ranges from 65% to 80%, depending on the level of metastatic spread.[2] Porocarcinoma is characterised by variable cellular atypia, increased mitotic count (including abnormal mitotic figures), necrosis and a focal ductal differentiation.[1]

While histological criteria aiding diagnosis are well established, data on the genetic foundations of these tumours are still very limited. Specifically, for poromas, loss of heterozygosity (LOH) of *APC* was reported more than a decade ago, and mutations in *HRAS* were described in two poromas only very recently.[4,5] Complementing a few reports on LOH of 17q and *APC* as well as *PIK3CA* activating mutations in porocarcinoma,[6–8] Harms *et al.* reported recurrent inactivating mutations in tumour suppressor genes *TP53* and *RB1* in five porocarcinomas, while mutations in *HRAS* and *EGFR* were less frequent.[5]

Extending this set of data, we analysed the mutational profile of poromas and porocarcinomas (six cases each) by high-coverage next generation sequencing (NGS) using a gene panel comprising 50 cancer-related genes.

MATERIAL AND METHODS

Study cohort and tumour samples

Poroma and porocarcinoma cases identified in the dermatopathology database of the Institute of Pathology, Faculty of Medicine, University of Belgrade, were retrieved from the archives and subsequently re-evaluated by two board-certified pathologists (DB and MB) according to the current World Health Organization (WHO) criteria.[3] Presence of ductal or intracytoplasmic lumina—demonstrated by haematoxylin and eosin (H&E) and immunohistochemical staining—and sharp demarcation from epidermal keratinocytes were necessary for the diagnosis in cases where no benign poroma component

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was observed.[1] Twelve cases (six poromas and six porocarcinomas) were found to be adequate (high tissue quality, i.e., no morphological artifacts, and minimum tumour cell content of 40%) for analysis by targeted next generation sequencing. Formalin fixed, paraffin embedded (FFPE) samples of these cases were transferred to the Center for Molecular Pathology, Institute of Pathology, University Hospital Heidelberg. Tissue was used in accordance with the regulations of the tissue bank and with the approval of the Institute of Pathology, Faculty of Medicine, University of Belgrade. Research was conducted in accordance with the principles of the Declaration of Helsinki.

DNA extraction from FFPE samples

For each tumour, areas were marked on an initial H&E stained slide and corresponding tumour areas were microdissected from subsequent unstained slides. Extraction of genomic DNA was performed by proteinase K digestion and fully automated purification using the automated Maxwell 16 Research extraction system (Promega, USA). The concentration of DNA was measured fluorometrically using the QuBit 2.0 DNA high sensitivity kit (Thermo Fisher Scientific, USA). Additionally, DNA concentrations and sequencing grade qualities were independently determined by a quantitative polymerase chain reaction (qPCR) assay (RNAseP assay; Thermo Fisher Scientific) as described previously.[9]

Library preparation and semiconductor sequencing

For library preparation, the multiplex PCR-based Ion Torrent AmpliSeq technology (Thermo Fisher Scientific), together with the Ion Ampliseq Cancer Hotspot Panel version 2 (CHPv2; Thermo Fisher Scientific) was used as described previously, enabling analysis of 50 cancer-related genes.[9–11]

Amplicon library preparation was performed with the Ion AmpliSeq Library Kit v2.0. The CHPv2 panel primer pool yields 207 amplicons. For amplification, approximately 10 ng of DNA, determined by qPCR assay, was used. Briefly, the DNA was mixed with the primer pool and the AmpliSeq HiFi Master Mix in a 20 µL reaction volume and transferred to a PCR cycler (Biometra, Germany). Subsequently, amplicons were partially digested using FuPa reagent, followed by the ligation of barcoded sequencing adapters (Ion Xpress Barcode Adapters; Thermo Fisher Scientific). The final library was purified using AMPure XP magnetic beads (Beckman Coulter, Germany) and quantified using qPCR (Ion Library Quantitation Kit; Thermo Fisher Scientific) on a StepOne Plus qPCR machine (Thermo Fisher Scientific). The individual libraries were diluted to a final concentration of 50 pM. Forty libraries were pooled and processed to library amplification on Ion Spheres using Ion Chef Ion 520 and Ion 530 Kit. Unenriched libraries were quality-controlled using Ion Sphere quality control measurement on a QuBit instrument. Pooling the libraries on a 530 chip resulted in a mean coverage of 2085-fold per amplicon.

Data analysis

Raw sequencing data were processed using the implemented Torrent Suite Software (version 5.2) and aligned against the human genome (version hg19) using the TMAP algorithm. For DNA mutation analysis, the aligned reads were processed using the build-in Variant Caller plugin (version 5.2). Only variants with a minimum coverage >100 reads were taken into account. Variant annotation was performed using a custom build variant annotation pipeline in the CLC Genomics Workbench (version 8.0.2). For visualisation of sequencing reads, the Integrative Genomics Viewer (IGV, http://www.broadinstitute.org/igv/) was used. The Catalogue of Somatic Mutations in Cancer (COSMIC) database was used for identification of already known somatic mutations and mutation types, respectively.[12] Copy number variations (CNVs; amplifications and deletions) were identified as described previously.[9,13]

RESULTS

Clinical and pathological characteristics

Table 1 summarises clinical and pathological information about patients with poroma (patients 1–6) and porocarcinoma (patients 7–12). Seven patients were female and five were male (age range 22–85 years, mean age 61 years). Tumours were localised on the legs in five (42%) patients, on the head in four (33%) patients, on the trunk in two (17%) patients, and on the arm in one patient (8%). Mean tumour diameter was 11 mm (range 5–19 mm). One poroma case was diagnosed as dermal duct tumour (patient 6), while the other five cases were classic poromas. None of the porocarcinomas showed a benign component, and two of the six cases displayed a malignant intraepidermal (*in situ*) component (patients 7 and 11). Mean thickness of porocarcinomas was 4 mm (range 2.7–5.3 mm). All patients who had porocarcinomas were diagnosed as localised disease, without metastasis in regional lymph nodes and distant metastases, and are still alive. Characteristic histological features of poroma (patient 1) and porocarcinoma (patient 10) are visualised in Fig. 1.

Genetic findings

Employing targeted high-coverage semiconductor-based NGS with a mean coverage of 2085 (range 149–5911) and mean total reads of 551,880 (range 467,250–678,477), we detected mutations in four of six poromas (67%) and four of six porocarcinomas (67%). Detailed results are presented in Table 2. Mutated poromas did not harbour more than one mutation per case, including two missense mutations in *HRAS* (p.G13R and p.Q61R) and one in *ERBB4* (p.E591K) and *APC* (p.P1433L), respectively. The latter mutations showed low allelic frequencies (5% and 3.57%, respectively) indicating subclonal events. All mutations were indexed in the COSMIC database.

Table 1 Basic clinicopathological characteristics of the cohort

Patient ID	Diagnosis	Gender	Age at diagnosis, years	Location	Tumour size, mm	Tumour thickness, mm
1	P	F	77	Arms, axilla	10	
2	P	M	43	Head, upper eyelid	7	
3	P	F	28	Legs, gluteal region	8	
4	P	F	65	Legs, shin	10	
5	P	F	61	Legs, dorsum of foot	5	
6	P	M	22	Legs, shin	7	
7	PC	F	68	Trunk, back	15	3.1
8	PC	F	76	Legs, shin	16	5.3
9	PC	F	80	Head, cheek	11	5.0
10	PC	M	76	Head, nose	10	4.9
11	PC	M	51	Trunk, abdomen	19	2.8
12	PC	M	85	Head, parietal region	14	2.7

P, poroma; PC, porocarcinoma.

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