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Original contribution

Porocarcinomas harbor recurrent *HRAS*-activating mutations and tumor suppressor inactivating mutations☆

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Summary Porocarcinomas are a rare eccrine carcinoma with significant metastatic potential. Oncogenic drivers of porocarcinomas have been underexplored, with *PIK3CA*-activating mutation reported in 1 case. We analyzed 5 porocarcinomas by next-generation sequencing using the DNA component of the Oncomine Comprehensive Assay, which provides data on copy number changes and mutational events in 126 cancer-relevant genes through multiplex polymerase chain reaction. We detected an average of 3.3 high-confidence nonsynonymous mutations per tumor (range, 1-6), including a spectrum of oncogenic activation and tumor suppressor inactivation events. Tumor suppressor mutations included *TP53* (4/5, 80%), *RB1* (3/5, 60%), *ATM* (2/5, 40%), *ARID1A* (1/5, 20%), and *CDKN2A* (1/5, 20%). In 4 (80%) of 5 tumors, at least 1 potential oncogenic driver was identified. Activating *HRAS* mutations were detected in 2 (40%) of 5, including G13D and Q61L hotspot mutations. Mutations of *EGFR* were identified in 2 (40%) of 5; these mutations have been previously reported in cancer but did not affect classic activation hotspot sites. *EGFR* and *HRAS* mutations were mutually exclusive. *HRAS* mutations were detected by targeted sequencing in a minority of benign eccrine poromas (2/17; 11.7%), suggesting that *HRAS* activation may rarely be an early event in sweat gland neoplasia. Together, our data suggest roles for *HRAS* and *EGFR* as drivers in a subset of poroma and porocarcinoma. *TP53* and *RB1* inactivation events are also likely to contribute to tumorigenesis. These findings suggest that porocarcinomas display diversity with respect to oncogenic drivers, which may have implications for targeted therapy in metastatic or unresectable cases.

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1. Introduction

Porocarcinomas are rare adnexal malignancies that display overlapping features with poromas or arise within a poroma. These tumors typically arise on extremities of the elderly but may present across a range of cutaneous sites and ages. Porocarcinomas have a 20% risk of metastasis to lymph nodes and/or distant sites [1,2]. Metastatic disease is often resistant to chemotherapy, and associated mortality is 67% [1,3].

Mechanisms of tumorigenesis in porocarcinomas remain poorly understood. *TP53* overexpression has been reported in most porocarcinomas suggesting dysregulation of this tumor suppressor, and *TP53* mutation has been identified in 1 case [4-7]. *PIK3CA*-activating mutation has been reported in 1 metastasizing porocarcinoma [8]. EGFR protein expression has been reported [8], but to our knowledge, the mutational status of this gene has not been examined in porocarcinoma. Therefore, oncogenic drivers have not been identified in most porocarcinomas.

Next-generation sequencing is a powerful approach to nominate potential drivers in rare malignancies. To better understand mutational changes in porocarcinoma, we examined the coding region of a panel of cancer-relevant genes by next-generation sequencing in a cohort of 5 porocarcinomas.

2. Materials and methods

2.1. Case selection

All studies were conducted under protocols previously approved by the Institutional Review Board at the University of Michigan Health System (UMHS). Porocarcinomas were identified by query of the UMHS Department of Pathology database over the period of 1999 to 2014 using the search term "porocarcinoma," and all available cases were retrieved. Diagnosis and adequacy were confirmed by review by a board-certified dermatopathologist (P. W. H. and/or R. M. P.). All cases displayed features of porocarcinoma including round basophilic cells and intermingled squamoid cells, foci of ductal differentiation, connection to the epidermis, mitotic activity, cytologic atypia, and infiltrative growth (Supplementary Figure S1). Although some cases displayed areas of squamous differentiation, there was no evidence of follicular or sebaceous differentiation in any case. Of 8 cases identified, 5 were suitable for sequencing (Supplementary Table S1), whereas 3 did not provide DNA of suitable quality.

2.2. Targeted next-generation sequencing

Hematoxylin and eosin–stained sections were used as a guide for dissection from a minimum of 4 formalin-fixed, paraffin-embedded (FFPE) 10-μm sections by a board-certified dermatopathologist (P. W. H.) to obtain a minimal tumor purity of 60%. DNA extraction, Ion Torrent–based next-generation sequencing, and data analysis were performed as previously described [9-12]. Briefly, DNA was extracted using the Qiagen FFPE AllPrep DNA/RNA kit (QIAgen, Germantown, MD). Bar-coded libraries were generated from 20 ng of DNA per sample using the Ion Ampliseq library kit 2.0 with the DNA component of the Ion Ampliseq Oncomine Cancer Panel primers targeting 126 cancer-relevant genes. Templates were prepared using the Ion PI Template OT2 200 Kit v3 (Life Technologies, Foster City, CA) on the Ion One Touch 2. Sequencing of multiplexed templates was performed using the Ion Torrent Proton on Ion PI Chip v2 using the Ion PI Sequencing 200 Kit v3 (Life Technologies) according to the manufacturer's instructions. Data analysis was performed using in-house developed, previously validated pipelines employing Torrent Suite 4.0.2, with alignment by Torrent Mapping Alignment Program and variant calling using the Torrent Variant Caller plugin. Annotated variants were filtered to remove synonymous or noncoding variants, poorly supported calls/sequencing artifacts, and germ line alterations. Variants present in the ESP6500 or phase I 1000G data set, the Exome Sequencing Project (ESP6500), or the Exome Aggregation Consortium at greater than 0.1% were filtered out as likely germ line alterations [13-15]. Potential driving alterations were prioritized using the COSMIC Database, Oncomine, and targeted literature searches. We have previously confirmed that these pipelines and filtering criteria identify variants that pass polymerase chain reaction (PCR) validation with greater than 95% accuracy [9-12,16,17]. For ultraviolet signature analyses, synonymous as well as nonsynonymous variants were considered.

2.3. *HRAS* mutation detection

To assess *HRAS* mutation status in poromas, we identified poromas by querying the UMHS Department of Pathology Database using the search term "poroma." Diagnosis and adequacy were confirmed by a board-certified dermatopathologist (P. W. H.). Genomic DNA was extracted from archival FFPE blocks using the QIAamp DNA FFPE Tissue Kit and quantitated by Qubit (Life Technologies). *HRAS* exons 2 and 3 were amplified by PCR using previously described primers [18], followed by Sanger sequencing at the University of Michigan Sequencing Core. Chromatograms were visualized using Sequence Scanner 2 software.

2.4. Immunohistochemistry

Rabbit anti-EGFR antibody (clone 5B7 predilute; Ventana Medical Systems, Tucson, AZ) and mouse anti-p53 antibody (clone DO7, predilute; Ventana Medical Systems) were used for immunohistochemistry on FFPE tumor sections using a Ventana automated stainer, following standard protocols including CC1 treatment. Staining patterns were evaluated by a board-certified dermatopathologist (P. W. H.). Expression of p53 was scored as absent, low (<25% of tumor cells), or high