Exploring the driver events of eccrine poromas and porocarcinomas: A retrospective, cross-institutional study of 54 cases

Dear Editor, Eccrine poroma (EP) and porocarcinoma (EPC) are rare benign and malignant adnexal neoplasms of the terminal sweat gland duct, respectively. Both can arise *de novo*, however, EPCs can also arise from pre-existing EP. To-date, our understanding of the genetics underlying these tumours has come from studies with small sample sizes and/or limited molecular analyses. [1-5] To comprehensively compare the driver events and mutational landscape of these tumours, we performed a retrospective multi-institutional whole-exome sequencing and RNA sequencing study on the largest cohort of EPs (n=31) and EPCs (n=23) to-date. The sequencing data has been released to the European Genome and Phenome Archive repository.

All EP and EPC tumours showed conventional histopathological features of poroma and porocarcinoma, including tumours with multifocal epidermal connections, extending in broad fronds into the dermis. They were composed of polygonal tumour cells with areas of duct differentiation. Additional features limited to porocarcinoma were infiltrative growth, marked cytological atypia and mitotic activity. The presence of ductal differentiation was confirmed in all tumours, to aid in distinguishing EPC from poorly differentiated SCC. No evidence of sebaceous differentiation was observed in any EP or EPC cases. We did not find any evidence of EPC cases arising in the background of an EP in this cohort. Follow up data was available for 22 EPC patients (median: 6 years post-diagnosis, range: 1-15 years post-diagnosis); 19 patients had no evidence of recurrence or metastasis, one patient died from a mass of unknown primary origin and two patients developed regional lymph node metastases (with one also developing lung metastases).

The tumour mutational burden of EPs was lower than EPCs (median = 0.207, range = 0.083 – 3.173 mutations/Mb versus median = 1.016, range = 0.165 – 88.356 mutations/Mb, respectively; Figure **1**). As shown in Figure **1 (a)**, EPs had recurrent mutations (n=2/31) in the cancer genes *TP53, NOTCH1* and *EGFR,* with the latter two not previously described, however they were not significantly mutated above the background mutation rate. In contrast, as shown in Figure **1(b)**, we identified four candidate driver genes (significantly mutated) in EPCs, specifically *TP53, HRAS, ZNF750* and *CIRBP*; the latter two not previously reported in EPCs. In addition, we show for the first time that the majority EPCs with *TP53* or *ZNF750* mutations show biallelic inactivation, due to loss of heterozygosity (LOH) of chromosome 17 or 17p/q, or copy neutral LOH of 17p/q. As shown in Figure **1 (b)**, mutational signatures SBS7a/b

(attributed to ultraviolet light) were identified in 10/23 EPCs, consistent with previous reports,[1,3] and in one EP (PD52507a). Notably, 6/13 of the EPCs with no UV signature were from the trunk, where exposure to damaging UV rays is less likely than the head and neck area or extremities, and only one EPC from the trunk was found to have a UV signature. Signatures SBS2 and/or SBS13 (attributed to AID/APOBEC activity) were identified in two EPCs, as previously reported.[3]

In contrast to EPs, EPCs showed significantly recurring somatic copy number (CN) gains and losses, consistent with previous reports.[3] Our large cohort size allowed identification of significantly recurrent focal amplification/deletion events, which represent candidate driver events. These included a 247kb amplification at 6q22.1 (encompassing the oncogene *ROS1* and *GOPC*, which is a fusion partner of *ROS1* in several cancer types) in two EPs and a 312kb deletion at 6p22.2 (encompassing *HFE*) in three EPs (Figure **1(a)**). In the EPCs, a 1.6Mb amplification at 11q13.3 (encompassing the oncogenes *CCND1, FGF3, FGF4* and *FGF19*) and a 6.9Mb deletion at 9p21.3 (encompassing the tumour suppressor genes *CDKN2A, CDKN2B, MTAP, TEK* and *MOB3B*) were each found in 7/21 samples (Figure **1(b)**).

As shown in Figure **1 (a)**, analysis of fusion genes in the EPs identified recurrent events involving *YAP1*. The predominant fusion partners were *NUTM1* or *MAML2*, as reported previously.[4] Interestingly, the *YAP1::MAML2* and *MAML2::YAP1* fusion genes were only found in the EP cohort, although they were previously reported in EPCs.[4] We also identified a novel fusion partner for *YAP1* (*CPEB3::YAP1*), and two novel *PAK1* fusions (*IGSF3::PAK1* and *DSC3::PAK1*). In contrast, as shown in Figure **1 (b)**, there was only one recurrent fusion in EPCs, specifically the previously reported *YAP1::NUTM1* fusion,[4] and a novel *HNRNPLL::YAP1* fusion in one EPC. The EPCs harboured several *PAK* family gene fusions, but no recurrence of a particular *PAK* fusion. Importantly, apart from *DLG1::PAK2*, these *PAK* fusions were different from those recently reported in a cohort of twelve EPCs.[5]

In summary, we have comprehensively characterised the mutational landscape of EPs and EPCs, uncovering novel events and delineating different pathways of tumourigenesis underlying these tumours. EPs are driven largely by oncogenic fusion genes, whereas EPCs are driven largely by somatic mutations affecting various pathways, with a subset driven by fusion genes as the first oncogenic driver, and somatic mutations representing secondary events contributing to progression of the tumour. Fusion genes in EP predominantly involve *YAP1* whereas those in EPC preferentially involve *PAK* gene family members.

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Figure legends:

Fig 1. Overall mutational landscape of eccrine poromas (EPs) and porocarcinomas (EPCs). (a) EP: the oncoplot panel shows OncoKB cancer genes for which a mutation was present in at least one sample. (b) EPC: the oncoplot panel shows genes previously reported as recurrently mutated in EPCs and/or those we identified as significantly mutated (those above the dashed line), and OncoKB cancer genes for which a mutation was present in at least four samples (those below the dashed line). Tumour mutational burden (TMB) is shown as

mutations per megabase. The focal copy number alterations (CNAs) shown are statistically significant. Abbreviations: LOH, loss of heterozygosity; sig, significant; UV, ultraviolet light. White asterisk indicates patients that developed metastases.