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Advancing toward a molecular characterization of Polymorphous Low Grade Adenocarcinoma

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Polymorphous low-grade adenocarcinoma (PLGA) is the second most common intraoral salivary gland malignancy; it affects predominantly the minor salivary glands of the palate, but has been reported to occur in the major salivary glands as well as extra palatal sites [1–3]. Despite the tendency for locally infiltrative growth, and the notable amount of locoregional recurrences, PLGA is characterized by an indolent clinical course with low risk of distant metastasis and good long-term prognosis [1, 3]. Nonetheless, given a wide diversity of histological architecture of PLGA tumors and their morphological overlap with other types of salivary gland tumors, their proper classification remains challenging and requires careful differential diagnosis with more aggressive tumors, such as adenoid cystic carcinoma [1]. As an example, in search of markers, the expression of *cox-2* has been evaluated in different salivary gland tumors' subtypes and demonstrated an association with the cellular composition of the subtypes, giving clues into the potential shared histogenesis of these tumors [4]. Additional markers to characterize these tumors' subtypes at a molecular level would be significant for their distinction.

A novel hotspot mutation in the *PRKD1* gene encoding serine/threonine kinase Protein Kinase D1 has recently been found to be highly recurrent in PLGA, with 72.9% of cases harboring c.2130A>T or c.2130A>C substitution [5]. PRKD1 plays a vital role in cell adhesion, viability, migration and survival via signal transduction in the diacylglycerol (DAG) and protein kinase C (PKC) pathway. The p.Glu710Asp mutation affects the kinase domain of PRKD1 and has been shown to be activating, resulting in increased cell viability

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and reduced cell migration. The mutation was not observed in non-PLGA salivary gland tumors [5].

We selected formalin fixed paraffin embedded (FFPE) tumors from patients who underwent therapeutic surgery and/or biopsy at The Johns Hopkins Hospital. A series of 14 slides was carefully reevaluated by an experienced pathologist (W.H.W.) to confirm the histological diagnosis (short fascicle and cribriform structures, targetoid arrangement of cords around blood vessels/nerves). To ensure >80% tumor cell content, tumor areas were marked and carefully dissected for DNA extraction. After xylene incubation for deparaffinization, DNA was extracted using conventional phenol/chloroform-ethanol precipitation method. Subsequently, genomic DNA was amplified by PCR with a specific primer pair designed by us [5'-TCCAACCTGGCTTTTGTGA-3' (forward) and 5'-AGATACTCGTGGCTTTGCGG-3' (reverse)]. The PCR products were analyzed on 1.5% agarose gel, purified and used for direct conventional Sanger sequencing.

13 tumors had sufficient and suitable DNA for analysis. 8/13 (61.5%) and 3/13 (23.1%) of the cases harbored c.2130A>C and c.2130A>T mutation, respectively, without any overlap. The remaining 2 tumors did not show *PRKD1* hotspot mutation. Representative sequences depicting the presence or absence of mutation are shown in Figure 1. Overall, *PRKD1* p.Glu710Asp mutation was present in 84.6% of PLGAs, confirming the high frequency previously reported. To our knowledge our study is the second to report a high prevalence of *PRKD1* mutation in PLGA.

Recently, a follow-up study reported that somatic mutations in other members of *PRKD* gene family members are not present in PLGA [6]. Interestingly, genomic rearrangements involving *PRKD1*, *PRKD2* and *PRKD3* genes have been implicated in pathogenesis of cribriform adenocarcinoma of minor salivary gland (CAMSG), an entity histologically related to PLGA [7]. These structural aberrations were also observed in tumors sharing CAMSG/PLGA morphology and in a single cases classified as a PLGA (*PRKD2* rearrangement), demonstrating difficulty of the exact discrimination of the tumor types basing solely on their histological features [7]. Interestingly, the two cases that did not show mutations in our study were the only ones classified as CAMSG. There has been a lot of controversy regarding the subclassification of PLGA and CAMSG [8–10]. Additional molecular studies in independent series of tumors may help to start shedding a light on this issue. Hotspot *PRKD1* mutation may thus constitute an important molecular marker to differentiate PLGA from other types (or subtypes) of salivary gland neoplasms, such as adenoid cystic carcinoma or pleomorphic adenoma. We believe that detection of this aberration could aid in the identification and lead to more precise diagnosis. Therefore, this alteration may become invaluable for clinical decisions in head and neck oncology.

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Figure 1. *PRKD1* mutation hotspots in Polymorphous Low Grade Adenocarcinoma (PLGA)
 Three representative sequences (electropherograms) of the *PRKD1* mutation hotspot and the region around it (hotspot c.2130 highlighted in pink). Upper panel shows wild type on case 3 (PLGA_3), middle panel shows a c.2130A>T mutation on case 8 (PLGA_8), lower panel shows a c.2130A>C on case 11 (PLGA_11).