

# The role of apical-basal polarity in oral cancer

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## Abstract

Over the last thirty years, improvements in survival rates of oral cancer patients have remained modest, hampered by the late diagnosis of the disease, a lack of understanding of the underlying biology of oral cancer, and a lack of identified actionable targets. While the apical-basal polarity has been widely investigated in normal and pathological contexts, its involvement in oral homeostasis is still not well understood. Here, we discuss the current documented role of PAR-3 complex-dependent apical-basal polarity regulation in oral cancer. We explore molecular switches that link polarity dysfunction to oral cancer initiation and highlight relevant models that would promote our understanding of disease development for therapeutic interventions.

## Introduction

### Oral squamous cell carcinoma

Head and neck squamous cell carcinoma (HNSCC) is the 6th most prevalent cancer worldwide, with more than half a million new cases reported annually [1,2]. Anatomical regions of SCC include the sinonasal and oral cavity, oropharynx, larynx, ear canal and trachea, with oral SCC (OSCC) being the most common malignancy of the head and neck region [3]. Patients diagnosed with OSCC have a particularly low five-year survival rate due to the compounding consequences of late detection [4,5]. Most OSCC patients usually present with advanced stage disease, and treatment is met with high levels of recurrence and metastasis [6,7]. In addition, OSCC patients continue to be at high risk of developing a second primary malignancy after their initial diagnosis [8,9]. The major risk factors for OSCC are tobacco use, alcohol abuse, betel nut chewing, genetic predisposition (eg. Fanconi anemia) and in the oropharyngeal anatomical subsite, infection with high-risk human papilloma viruses (HPV) [3,10]. Based on these differing aetiologies, it is hardly surprising at a molecular level that OSCC is a highly heterogeneous disease [11-14].

HPV-related oropharyngeal SCC are still diagnosed at lesser rates than HPV-negative OSCC and the prevalence of high-risk HPV positivity in OSCC varies widely between developing countries [1]. In the HPV-negative subsets, chronic exposures to carcinogens and extensive consumption of alcohol have been largely associated with the induction of neoplasms [1,15-17]. Oral leukoplakia and oral lichen planus are among the most frequent oral potentially malignant lesions and patients with those lesions are more susceptible to developing OSCC [18-21]. Comparably, immunosuppressed transplanted individuals are also prone to OSCC [22] and a high occurrence of oral cancer has been noted following post haematopoietic stem cell transplantation and liver transplant patients [23,24]. Despite those correlative studies, significant insights into the molecular mechanisms that initiate OSCC are still lacking. Therefore, defining the key effectors

in oral homeostasis is an urgent clinical need with potentials to open-up novel avenues for early detection of precancerous lesions, providing a scaffold for the development of survival improving preventative and therapeutic interventions against OSCC.

### Oral homeostasis

The oral mucosa of the tongue consists of a connective tissue known as the lamina propria covered by squamous stratified cells forming the oral epithelium (OE). Stem and progenitor cells in the basal layer of the OE divide to repair any transient damage and to maintain rapid self-renewal of the tissue [25,26]. Upon commitment to differentiation, similar to skin keratinocytes, the proliferating cells polarize and migrate upward to form consecutively, the spinous, granular, clear and cornified layers [27,28]. The last step of this differentiation program establishes the cornified layer that provides a functional protective outer barrier in the dorsal tongue. In addition, terminally differentiated cells produce anti-microbial peptides that contribute to the protective response against any epithelial damage [29,30].

Mutations in genes that regulate squamous differentiation were observed with high rates in oral cancers [12,13,31]. This suggests that oral homeostasis is tightly controlled by regulatory mechanisms of differentiation implicating dysregulation of this process as a driver of OSCC. It is well recognized that regulation of polarized protein segregation governs cell division, migration and differentiation, while

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disruption in these processes results in barrier function impairment, epithelial hyperproliferation and in some cases, SCC [32-35]. Important modulators of those mechanisms are apical-basal polarity complexes that have been shown to modulate keratinocyte differentiation and epithelial barrier function [36,37]. Therefore, the regulation of polarity proteins is essential for the normal function of stratified epithelia and for the maintenance of their homeostasis.

**Apical-basal polarity in oral cancer**

Different polarity complexes mark front, rear, apical, basal and adjacent sides of the cell allowing basal cells to self-replenish and remain in the basal layer while progenitor cells migrate towards the surface of the stratified epithelium. The Par-3/Par-6/aPKC/Cdc42 is an important polarity complex that contributes to those highly regulated processes by initiating the conversion of adherens junctions (AJ) to form “belt-like” junctions in preparation of epithelial cell polarization [38]. Gödde *et al.*, has detailed the role of the Par-3 complex in polarity and cancer in multiple murine models showing that loss of PAR-3 alone is not sufficient to induce malignant transformation, but rather requires additional oncogenic events [39]. In mammary tissues, ductal hyperplasia of Par-3- deficient cells did not progress to malignancy [40]. However, in various breast cancer mouse models, it was only following the induction of oncogenic hits that the tissues transformed to cancer, albeit at a faster progression rate in the absence of PAR-3 [41,42]. Furthermore, with regards to squamous epithelia, the polarity protein PAR-3 was shown to control epidermal homeostasis through regulation of barrier function, keratinocyte differentiation, and stem cell maintenance [43].

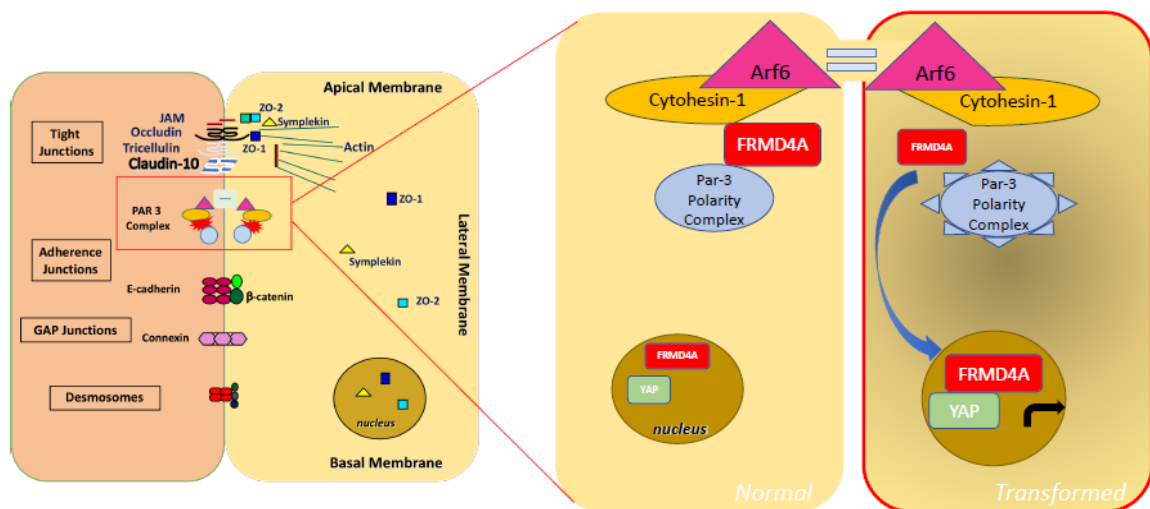
Nevertheless, and despite its established function in the skin, little is known about the role of core components of the Par-3 complex in squamous tissue homeostasis. A genome-wide screen of 684 human cancer cell lines revealed homologous PAR-3 exon microdeletions occurring predominantly in SCC, including OSCC. In addition, the loss of tight junctions in lung SCC is observed at the expense of PAR-3 deletion or suppression while the upregulation of yes- associated protein (YAP1) and aPKC were noted in OSCC and HNSCC, respectively [44,45].

Because the Par-3 complex is involved in the sequential stratification of the oral epithelium [46,47] and since OSCC are

characterized by disrupted differentiation and stratification, it is reasonably predictable that oral cells may have lost their ability to recognize apical-basal polarity at the initial stage of transformation. Therefore, through analyses of multiple PAR-3 partners and regulators, mechanistic insights into the function of Par-3 polarity complex could be seen shedding light on OSCC initiation.

**FERM domain containing 4A (Frmd4a)**

The FERM family of ezrin, radixin and moesin has been identified as integral for communication and transportation between the cytoskeleton and the plasma membrane, and co-localize with the Par-3/Par-6/aPKC/Cdc42 complex [48]. The FERM proteins facilitate the linking of transmembrane proteins such as CD44, CD43, I-CAM2 and I-CAM3 within the intercellular space [49-53]. Interestingly, of the eight currently identified FERM proteins [54] only FRMD4a has been shown to play a role in the regulation of apical-basal polarity [38], oncogenesis [55,56] and interaction with tumor promoting factors in OSCC [57]. FRMD4a is known to regulate the apical-basal polarity by linking the Par-3 complex with the guanine nucleotide exchange factor of Arf6, cytohesin-1, which is necessary for Arf6 activation consequently maintaining epithelial polarization (Figure 1) [38]. While FRMD4a is mainly expressed in basal cells of the oral stratified squamous epithelium, its expression is found reduced with differentiation with no signal detected in differentiated and terminally differentiated layers of the normal tissue [38]. On the other hand, FRMD4a is recognized as a stem cell marker in normal oral cells, and knockdown of Frmd4a either in vitro or in xenografts correlates with E-cadherin downregulation and subsequent reduction in cell proliferation. Furthermore, in situ hybridization of Frmd4a in OSCC sections demonstrated increased expression that is no more limited to basal cells [54] and prominent mRNA and protein expression of this stem cell factor were found in all OSCC lines tested [58,59]. Goldie *et al.*, also noted a direct correlation between increased FRMD4a expression and the risk of OSCC recurrence in two retrospective data analyses [54]. Moreover, Zheng *et al.*, complemented these findings and reported that there is a significant correlation between the overexpression of FRMD4a and the rate of OSCC metastasis to lymph nodes [55]. More interestingly, increased nuclear localization of YAP was associated with nuclear FRMD4a expression in SCC cells suggesting that FRMD4a may influence the Hippo pathway, dysregulating growth control and oral homeostasis



**Figure 1.** Schematic representation of FRMD4a interaction with the Par-3 polarity complex in the cytoplasm of normal cells and its association with YAP in the nucleus of transformed cells

[54,59]. In earlier studies, FRMD4a was also shown to bind to YEATS domain containing 4 (aka YEATS4, Gas41), which is upregulated in tumor cells and predominantly expressed in the nucleoli [56]. Therefore, in a normal situation, cytoplasmic FRMD4a contributes through the Par-3 complex to apical-basal polarity, while the dissociation of FRMD4a and its relocalisation to the nucleus seems essential to initiate dysplastic transformations and to drive tumorigenesis (Figure 1). These data underline a merit to further explore the biological function of FRMD4a whereby future experiments could pioneer novel therapeutic approaches by either preventing FRMD4a nuclear translocation or by targeting its downstream oncogenic function in oral cancer.

## Conclusion

Our understanding of how the Par-3 apical-basal polarity complex influences oral differentiation, polarization, stratification and cancer development, whether through core components or binding partners, remains currently limited due to the lack of relevant laboratory and animal models. Future studies should be conducted to determine how FRMD4a interacts with PAR-3 in normal tissues and how FRMD4a dissociation and mis-localization to the nucleus could engage the Hippo-YAP signaling activation in the initiation of epithelial transformation, particularly in OSCC.

## Conflict of interest

The authors declare no conflict of interest.

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