RESEARCH HIGHLIGHT

Role of Epidermal growth factor receptor in odontogenic epithelium and development of odontogenic lesions

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Growth is a highly coordinated process which is sustained by several growth factors and apoptotic factors. Any disturbance in this delicate balance leads to pathologies and genes that have such potential to produce tumors when mutated are known as oncogenes. EGFR an important growth factor that is involved in several physiological processes is presently one of the most common genes in targeted cancer therapies. Its potential as an oncogene target in squamous cell carcinomas of the head and neck epithelial tumors is gaining importance leading to revolutionization of cancer treatment modalities, its role in other head and neck epithelia like odontogenic epithelia remains vague and needs attention. The present article highlights some of the key findings in our research evaluating the role of EGFR in physiologic odontogenic epithelium that is comprised within pericoronal follicles. The research involved study of immunohistochemical examination of 35 pericoronal follicles removed from patients with asymptomatic impacted tooth extractions. The follicles were assessed for intensity, percentage of staining and location of the EGFR stain. The follicles predominantly showed intense staining pattern and location of EGFR positivity in most epithelium and rests were combined both cytoplasmic and membrane positivity. These findings reemphasize the inherent proliferative potential present in follicles and their role in formation of odontogenic tumors like ameloblastomas in long term impacted teeth. The potential of EGFR as a treatment target in odontogenic tumors also remains plausible.

Keywords: lipopolysaccharide; immune-brain; neurodevelopment; sex differences; elevated plus maze; open field; light/dark test; neonatal; microglia

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Cell surface receptors play a critical role in transmitting extracellular environment information with the internal milieu. One of the major family of transmembrane proteins playing a role in this critical communication is the HER family or the ErbB family of receptors which possess the intrinsic protein tyrosine kinase activity [1]. EGFR is the archetypal member of this family being the first to be sequenced as well as known to have tyrosine kinase activity. EGFR structure consists of one polypeptide chain consisting of 1186 amino acid residues weighing 170kDa with three domains; the extracellular, transmembrane and the intracellular domains containing tyrosine kinase enzyme [1]. The extracellular domain is the ligand bind domain and mainly binds epidermal growth factor (EGF) and other similar ligands like TGF-β, hepatocyte growth factor (HGF) and neuregulins [2]. On binding of the specific ligand they form a homo- or heterodimeric complex, following which there is internalization of the receptor-ligand complex resulting in autophosphorylation of the tyrosine which activates an internal cascade of signaling pathways that

mediate various cellular functions like determination of cell lineage, cellular proliferation, cell homeostasis, organ morphogenesis, cellular motility and cell survival [3].

This receptor pathway may be disrupted by changes like gene mutation, chromosomal translocations, gene amplifications and altered ligand production which may lead to disruption or persistent activation of the EGFR. The EGFR gene when disrupted produces receptor proteins which results in abnormal cell growth or tumorigenesis [3,4]. This gives EGFR an oncogene status as it has the ability to initiate carcinogenesis when it is altered. EGFR has been reported to overexpress in numerous carcinomas like head and neck squamous cell carcinoma (HNSCC), non-small cell lung cancer (NSCLC), colorectal, ovarian, breast carcinoma, prostate and renal cancers and has been correlated with tumor progression, resistance to conventional therapy and poor prognosis [1,3,4]. Even though carcinomas represent a very complex process as a result of several genetic and epigenetic changes over the years, it is now known that by reversing or blocking few critical pathways it is possible to reverse the fate of the tumor. Weinstein *et al* described this dependency of a malignant tumor on critical genes for its survival as oncogene addiction and they suggested that by understanding this state, we can target the weak point of the tumor process using monotherapy or combination therapy against these oncogenes.[4] EGFR remains one of the most targeted oncogenes with several anti - EGFR agents like cetuximab, gefitinib, erlotinib in monotherapy and combination being used in several carcinomas HNSCC, colorectal, NSCLC and pancreas [5,6].

Head and neck epithelia represent a distinct type of epithelium with a predominant contribution from the neural crest cells along with the ectodermal germ layer. EGFR is proven to have a significant function in the development and maintenance of various oral structures. The increased expression of EGFR mRNA and protein in approximately 90% of HNSCC leads to the increased interest in knowing about the EGFR in other head and neck epithelia like the salivary gland epithelia and odontogenic epithelium [5,6,7,8]. Several studies have also previously confirmed the role of EGFR in tooth development, eruption and morphogenesis [7-10].

Odontogenesis is a highly coordinated process which results from a series of interactions involving the oral ectoderm and the neural crest cells referred to as ectomesenchyme [11-13]. The dental lamina remnants, reduced enamel epithelium and ectomesenchyme persist above the crown of unerupted and impacted teeth, where it is referred to as pericoronal follicle (PF) or dental follicle (DF). The sources of odontogenic epithelium hence includes the dental lamina remnants otherwise known of cell rests of Serre, rests of Malassez, reduced enamel epithelium and the basal cells of the oral epithelium which are usually in a state of inactivity or rest and due to some unknown stimuli they have the potential to proliferate into various odontogenic cysts and tumors [2,7,11]. Researchers who have previously studied the EGF and EGFR have suggested that regulation of EGF-R expression is developmentally determined in human odontogenesis. Wise *et al* [12] and Cadhill *et al* [9] have elicited presence of EGFR in tooth follicle establishing its role in tooth eruption. EGFR and its ligands were also found to play a role in tooth morphogenesis as claimed by a study Hu *et al* [8]. The odontogenic epithelium is the main target tissue for EGF, TGF-β and TGF-α and is speculated to also be involved in odontogenic tumorigenesis.

The present paper highlights some of the important finding that were derived on assessment of EGFR expression in physiologic odontogenic epithelia as represented by the pericoronal follicle which usually has remnants of the lamina (cell rests of Serre) and the reduced enamel epithelium, and its possible importance in future treatment strategies involving molecular targets like EGFR and its downstream players [13].

All follicles demonstrated 100% EGFR expression, confirming that the odontogenic epithelium has EGF receptors and it is susceptible to the presence of EGF and associated ligands. It was noted that the EGFR stained both the lining epithelium and rests intensely in most follicles showing that they have the potential to undergo proliferation in suitable conditions. It was also noted that the odontogenic rests marginally stain with more intensity than the reduced enamel epithelium suggesting that the dental lamina remanants are more responsive to EGF ligands. These data suggest and reconfirm that impacted teeth and follicles should be removed as a precautionary measure.

The extent of staining in our study was assessed by measuring percentage of cells with EGFR expression it was found that majority of the follicles (54%) showed greater than 50% of cells with EGFR positivity. It was further noted that in the smaller odontogenic rests intense staining with 100% positivity was noted whereas in the bigger rests it was seen than the more central cells resisted EGFR staining similar to the findings of Christensen *et al* in keratin pearls in squamous cell carcinoma [14]. The author hypothesized that this phenomenon suggests that EGFR is present more on undifferentiated cells and its extent varies inversely with cellular differentiation [14].

The localization of EGFR has acquired a lot of interest as this parameter denotes a lot about the biologic behavior of

the tissue. In the present study, analysis of normal oral epithelium used as positive control showed intense combined cytoplasmic and membrane expression in the basal and parabasal layers, while staining limited to the membrane was visible in the spinous layers, which further decreased to no expression in the upper mature layers. Squamous cell carcinoma controls on the other hand showed mostly intense membrane and combined staining patterns. This expression observed in our study is similar to that obtained by Maiorano *et al* who documented similar findings in oral mucous membrane and associated membrane expression with better prognosis in squamous cell carcinoma.[15] In contrast, recent studies by Da silva Baumgart *et al* and de Oliveira *et al* showed all normal oral mucosa fields presenting with a combined staining pattern symbolic of slower proliferative response and membrane staining was strictly associated with high proliferation [16,17].

In our study, follicles predominantly showed combined (cytoplasmic and membrane) staining pattern (40%) representing increased proliferative potential similar to squamous cell carcinoma and the basal and parabasal layers of oral mucosa. Follicles with cytoplasm only staining could represent quiescent cells which have the potential to proliferate in the presence of growth factors or they could represent cells which, after differentiation, EGFR is internalized and degraded. Membrane-only staining, on the other hand, may represent an active proliferative cell or a well differentiated cell with decreased or increased proliferative potential depending on the stimuli. In squamous cell carcinomas the former may be true as intense membrane staining is common, as mutations causes upregulation of EGFR receptor on the tumor cells helping in their increased proliferation. On the other hand membrane staining may also be found in physiologic situations like mature cells in the spinous layers of oral mucous membrane, tall columnar ameloblastic cells of reduced enamel epithelium, squamous differentiation in odontogenic rests etc may have increased or decreased proliferative potential depending upon availability of ligands. In contrast, Baumgart *et al* in his study on pericoronal follicles associates cytoplasmic EGFR labeling is with slower proliferation due to internalization of the EGF receptor [16]. Combined cell and membrane positivity was related to a more physiologic-type response and membrane only response was associated with greater proliferative potential. Epithelium and rests with squamous metaplasia showed mostly membrane only staining, which indicates the possibility that for squamous metaplasia the EGFR is externalized and used. It was also noted that nests showing squamous differentiation showed more membranous patterns in the center, which is also similar to normal oral mucosa. Thus, in the present study, in physiologic odontogenic epithelium, membrane only staining has been correlated with more mature cells which have low proliferative potential or undergo squamous differentiation. Several other studies have also documented that squamous metaplasia in pericoronal follicles could represent early changes in the development of odontogenic lesions [17]. In the reduced enamel epithelium containing tall columnar ameloblastic cells, an intense to mild membrane only staining was observed, suggestive that EGFR is responsible in the ameloblastic differentiation and plays a role in amelogenesis. In other studies on ameloblastomas also, it was noted that the peripheral tall columnar cells and pre-ameloblasts had a membranous staining pattern [18,19]. These findings suggest that understanding EGFR stain location plays a vital role in assessing its proliferative potential, biological aggressiveness and treatment options.

In conclusion, it is noted that PFs showed predominantly intense combined and cytoplasmic staining patterns, suggestive of an inherent potential for proliferation in dental follicles. Finally, the areas of squamous metaplasia showed a consistent membrane EGFR expression, which highlights the role of EGFR in squamous differentiation and may be related to early pathological changes in dental follicles. Ameloblastomas and odontogenic cysts like keratocystic odontogenic tumor and dentigerous cysts are often derived from the epithelial remnants and follicles and this suggests that early intervention of removing the impacted teeth and associated follicles can reduce pathologies to a certain extent. It is also hypothesized that ameloblastomas can be reduced in size and recurrence can be prevented by using anti- EGFR agents as several studies on ameloblastoma molecular pathways suggest that several downstream markers a show increased EGFR immunohistochemical expression and mutations [18]. Immunohistochemical reactivity for EGFR downstream markers like BRAF, K-Ras, MEK1, Raf1, and ERK1/2 have been identified in both normal odontogenic epithelium as well in odontogenic tumors [4], however, further insight is necessary for adopting ideal treatment strategies for odontogenic pathologies.

Conflicting interests

The authors have declared that no competing interests exist.

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