Molecular and Clinical Aspects of Drug-induced Gingival Overgrowth

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Abstract

Drug-induced gingival overgrowth is a tissue-specific condition and is estimated to affect approximately one million North Americans. Lesions occur principally as side-effects from phenytoin, nifedipine, or ciclosporin therapy in approximately half of the people who take these agents. Due to new indications for these drugs, their use continues to grow. Here, we review the molecular and cellular characteristics of human gingival overgrowth lesions and highlight how they differ considerably as a function of the causative drug. Analyses of molecular signaling pathways in cultured human gingival fibroblasts have provided evidence for their unique aspects compared with fibroblasts from the lung and kidney. These findings provide insights into both the basis for tissue specificity and into possible therapeutic opportunities which are reviewed here. Although ciclosporin-induced gingival overgrowth lesions exhibit principally the presence of inflammation and little fibrosis, nifedipine- and especially phenytoin-induced lesions are highly fibrotic. The increased expression of markers of gingival fibrosis, particularly CCN2 [also known as connective tissue growth factor (CTGF)], markers of epithelial to mesenchymal transition, and more recently periostin and members of the lysyl oxidase family of enzymes have been documented in phenytoin or nifedipine lesions. Some oral fibrotic conditions such as leukoplakia and oral submucous fibrosis, after subsequent additional genetic damage, can develop into oral cancer. Since many pathways are shared, the study of gingival fibrosis and comparisons with characteristics and molecular drivers of oral cancer would likely enhance understandings and functional roles of molecular drivers of these oral pathologies.

Keywords: gingiva, cell signaling, connective tissue biology, inflammation, collagen(s), innate immunity

Introduction

Drug-induced gingival overgrowth is tissue-specific and results principally from medical therapy with the anti-seizure medication phenytoin, the anti-hypertensive drug nifedipine, and the immune suppressor ciclosporin (formerly known as “cyclosporin A” or “Cyclosporine”). More than 3 million Americans suffer from seizure disorders, 20% of whom remain on phenytoin administration principally due to grand mal epilepsy (Shimada and Takemiya 2014). Sales figures for generic and non-generic nifedipine tablets that are used for treating hypertension were \$143 million in 2007 in North America, enough to provide 500,000 people with a full year of medication, while worldwide sales were \$1.2 billion dollars in 2010 and 2011, respectively (http://www.evaluategroup.com/View/3270-1003-modData/generic_name/nifedipine). Similarly, ciclosporin therapy remains prescribed for a significant proportion of organ transplant and autoimmune patients, with worldwide sales at \$1.6 billion in 2010 and 2011, respectively, and an annual growth of 3.5% (http://www.evaluategroup.com/View/12460-1003-modData/generic_name/ciclosporin). Expanding indications for which ciclosporin therapy is effective were recently highlighted (Wright et al. 2005), demonstrating that overall usage of ciclosporin will not decrease in the foreseeable future. These newer indications include, but are not limited to, cardiac and liver transplantation in children, treatment of Behçet’s disease, pemphigus vulgaris, rheumatoid arthritis, active

hepatitis, and AIDS (Wright et al. 2005). Thus, it is apparent that at least two million North Americans are at risk for drug-induced gingival overgrowth. While varying in different populations, the incidence of gingival overgrowth from each of these drug therapies can be up to 50%. Thus, currently one million residents of North America are likely to suffer from drug-induced gingival overgrowth.

Gingival overgrowth presents major problems for the maintenance of oral hygiene. Increased swelling and disfiguration of gingiva elevate the risk for infection and inflammatory complications in patients who have undergone solid organ transplantation or who have epilepsy or cardiovascular diseases. Additional ramifications of gingival overgrowth include difficulty with mastication and a disfigured appearance. Non-surgical approaches can reduce the size of the clinical lesions by up to 40%, mainly due to the elimination of bacteria. The fibrotic enlargement remains, accounts for the 60% of the

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overgrowth, and requires surgical intervention (Kantarci et al. 1999). In most cases of severe gingival overgrowth, drug therapies cannot be altered; lesions recur after surgical excision, and require repeated surgical interventions. Evidence suggests that 34% of cases demonstrate recurrence during the 18 months following periodontal surgery regardless of the drug (Ilgenli et al. 1999). While some surgical approaches, such as the use of laser excision, reportedly reduce the recurrence compared with the conventional gingivectomy or modified flap techniques (Mavrogiannis et al. 2006), re-growth of the excised gingival tissue due to the continuous use of the drug presents a significant challenge in clinical practice. Although gingival overgrowth lesions are not directly life-threatening and may be tolerated by some patients without treatment, the quality of life is clearly compromised among affected individuals. Reliable quantitative data distinguishing the prevalence of severe vs mild drug-induced gingival overgrowth and their long-term prognoses are not available. Pharmacologic strategies, such as the use of the antibiotic azithromycin, are being studied, mainly due to their direct effects on human gingival fibroblasts, but no clear understanding of the underlying mechanisms has been reported (Citterio et al. 2001; Paik et al. 2004; Kamemoto et al. 2009). Non-surgical therapies to treat gingival overgrowth and stabilize the long-term outcomes are needed to alleviate suffering for those who are adversely affected. In addition to drug-induced gingival overgrowth, inherited and idiopathic forms of this condition, while rare, occur and have been reviewed previously (Hassell and Hefti 1991; Marshall and Bartold 1999; Seymour et al. 2000; Trackman and Kantarci 2004; Bharti and Bansal 2013). Although progress in the clinical management of human gingival overgrowth has been made in relatively affluent societies, approaches of drug substitutions and careful dose adjustments are not universally practiced by physicians worldwide, and this contributes to the global public health impact of gingival overgrowth (Bharti and Bansal 2013).

There is a critical need for better understanding of the tissue, cellular, and molecular drivers of the tissue specificity of gingival overgrowth to develop specific strategies to reduce its occurrence. As already amply summarized by us and others, gingival overgrowth leads to compromised quality of life for patients, and can lead to indirect negative effects on systemic health (Hassell and Hefti 1991; Marshall and Bartold 1999; Seymour et al. 2000; Trackman and Kantarci 2004; Wright et al. 2005; Bharti and Bansal 2013). Present and future novel cellular mechanisms discovered in analyses of gingival overgrowth may have relevance to other oral diseases, most notably oral cancer, and neurofibromatosis-1, with the latter often accompanied by gingival overgrowth and tongue lesions which can progress to oral cancer (Cunha et al. 2004; Jouhilahti et al. 2012). The purpose of the current review is to call attention to molecular aspects of gingival overgrowth to begin to identify pre-clinical approaches that may ultimately be useful for the prevention or treatment of gingival overgrowth and which may have relevance to other pathologies of the oral cavity, such as fibrosis associated with oral cancers.

Figure 1. Relationship among inflammation, fibrosis, and drug treatments which cause gingival overgrowth in humans. The left side of represents high fibrosis which is accompanied by high CCN2 expression, while the right side represents high inflammation and low fibrosis (Uzel et al. 2001).

Are All Drug-induced Forms of Gingival Overgrowth Fibrotic?

The cellular and tissue characteristics of human gingival overgrowth lesions caused by phenytoin, nifedipine, and ciclosporin, respectively, have different characteristics. A careful analysis of human gingivectomy samples comparing cellular and molecular features provided compelling evidence that lesions differ as a function of drug treatment. Phenytoin-induced lesions are clearly the most fibrotic, ciclosporin-induced lesions are highly inflamed and exhibit little fibrosis, while nifedipine-induced lesions are mixed (Fig. 1) (Uzel et al. 2001). Findings were based on histological and histomorphometric analyses. Expression in phenytoin-induced gingival overgrowth of TGF- β and CCN2 (connective tissue growth factor), which induce extracellular matrix synthesis and accumulation, was elevated. Mesenchymal cell proliferation was increased in tissues which exhibited gingival overgrowth, while apoptosis was diminished, especially in phenytoin-induced gingival overgrowth (Hong et al. 1999; Uzel et al. 2001; Kantarci et al. 2007; Thompson et al. 2010). A more recent study has identified the fibrosis marker periostin to be up-regulated in nifedipine-induced gingival overgrowth (Kim et al. 2013). Nifedipine was observed to, in some way, enhance TGF- β signaling, which leads to increased periostin expression (Kim et al. 2013). Periostin, like CCN2, is a matricellular protein and contributor to fibrosis. Periostin has been reported to enhance extracellular matrix biosynthesis through its interactions with the procollagen-C-proteinase BMP1 protein, which processes many important extracellular matrix protein precursors to mature functional extracellular matrix structures (Maruhashi et al. 2010; Muir and Greenspan, 2011). To our knowledge, the presence of periostin has not been evaluated in any other forms of human gingival overgrowth.

The finding of low fibrosis and high inflammation in human ciclosporin-induced gingival overgrowth (Uzel et al. 2001) is in contrast to a widely held perception that all forms of gingival overgrowth are fibrotic (Morton and Dongari-Bagtzoglou 1999; Kataoka et al. 2000; Wright et al. 2005; Chiu et al. 2009; Chin et al. 2011; Kuo et al. 2012). Mechanisms by which ciclosporin induces high inflammation and low fibrosis are unclear. It may seem counter-intuitive that ciclosporin therapy results in oral tissues that are primarily inflamed with little fibrosis, while kidney fibrosis is a severe complication of ciclosporin

therapy. (For reviews, see Busauschina et al. [2004], Hesselink et al. [2010], and López-Hernández and López-Novoa [2012].) The innate immune system is particularly important in the oral environment, due to microbial and physical insults which are not as relevant to internal organs such as the kidney. The innate immune system is normally in balance with the acquired immune system (Crawford et al. 1994). Moreover, ciclosporin targets cyclophilin and inhibits IL-2 stimulated T-cell production, attenuating the acquired immune system (Borel, 1991). The oral environment in which the innate system is highly stimulated may be particularly sensitive to the absence of the dampening effects of the acquired immune system. Inhibition of cyclophilin by ciclosporin disrupts collagen deposition and maturation in part by inhibition prolyl-3-hydroxylase activity (Steinmann et al. 1991; Cabral et al. 2014). Hydroxylation of collagen on proline residues by proline-3 hydroxylase and the more prevalent proline 4-hydroxylase facilitates formation of the characteristic collagen triple-helical structure in the endoplasmic reticulum during its biosynthesis. Thus, mechanisms of ciclosporin to promote inflammation and limit fibrosis in oral tissues may involve both an exaggerated innate immune response and the additional anti-fibrotic effects of ciclosporin A on collagen biosynthesis and deposition.

Fully consistent with the observed differences in the balance of inflammation and fibrosis between ciclosporin- and phenytoin-induced gingival overgrowth (Uzel et al. 2001) is a study in which the expression of immune modulators was investigated as a function of exposure of gingival fibroblasts to Toll-like receptor (TLR) ligands (Suzuki et al. 2009). Analysis of the data showed that treatment of human gingival fibroblasts with ciclosporin alone did not induce inflammatory responses. However, expression of the inflammatory markers IL-6, IL-8, and CD54 was elevated in the presence of both ciclosporin plus TLR ligands above the levels of TLR ligand treatment alone. By contrast, phenytoin treatment alone or in combination with TLR ligands inhibited expression of these inflammatory markers, possibly consistent with the low inflammation observed in human gingival overgrowth. In vivo, the same study found that ciclosporin enhanced the expression of CD54 in mice treated with the TLR ligand lipopolysaccharide, a finding which supports the in vitro data summarized above (Suzuki et al. 2009).

Analyses of balances between cell proliferation and apoptosis which assessed for the expression levels of molecular markers in human lesions revealed enhanced proliferation and reduced apoptosis in all forms of drug-induced gingival overgrowth. Particularly strong increases were observed in the percent of apoptotic cells (apoptotic index) in the gingival sulcus of control individuals which correlated positively with the degree of inflammation at this site. The sulcus is a region of the gingiva which experiences a relatively high degree of inflammation compared with that in the gingiva adjacent to the oral epithelium. In gingiva from all drug-related subjects, inflammation-associated apoptosis was attenuated at all sites, and proliferation was enhanced (Kantarci et al. 2007). These studies emphasize the hyperplastic nature of gingival overgrowth, and suggests that the identity and quantity of inflammatory cells in gingival tissues likely differ as a function of the causative drug, as evidenced by quantitative analyses of differences in

the relative degrees of apoptosis and inflammation and extracellular matrix accumulation (fibrosis) between the different drug treatments (Uzel et al. 2001; Kantarci et al. 2007). Thus, although the general trends toward decreased apoptosis and increased proliferation are common among all forms of gingival overgrowth, the degree and possibly the nature of the inflammation differ. These differences are reflected in quantitative comparisons and in the differences in the nature of the lesions as a function of drug treatment.

As noted, the degree of inflammation is highly elevated in ciclosporin-induced gingival overgrowth. It is therefore reasonable to suspect that the innate immune response becomes abnormally high in the ciclosporin-inhibited acquired immune response, as suggested above. Support for immune system modulation in gingival overgrowth comes from studies of the balance between levels of inflammatory macrophages, and synthetic macrophages in phenytoin-induced overgrowth indicated that phenytoin caused a shift to synthetic macrophages in areas of tissues which were inflamed, compared with inflamed tissues from individuals not treated with phenytoin (Iacopino et al. 1997). In addition, the phenytoin-treated individuals exhibited high levels of PDGF B expression. Subsequent studies of nifedipine- and ciclosporin-treated individuals have also found altered macrophage balances as a function of drug treatment (Pernu and Knuuttila 2001; Nurmenniemi et al. 2002). Data analysis indicated elevated epithelial content of inflammatory macrophages expressing the 27E10 antigen, which also marks monocytes. Synthetic RM3/1-positive cells were higher in the connective tissue of immunosuppressed individuals, while cells expressing antigen 25F9-positive macrophages were at lower levels. Antigen 25F9 is a marker of macrophage differentiation, and the authors suggested that, in immunosuppressed patients, gingival macrophage development is delayed, consistent with the notion expressed above which postulates that an attenuated acquired immune response has effects on innate immune system activity (Nurmenniemi et al. 2002). Taken together, these findings point to the importance of dysregulation of interactions between the innate and acquired immune systems in gingival overgrowth, which may occur to various degrees in different drug-induced forms of gingival overgrowth.

An interesting in vitro study of the effect of nifedipine on gingival fibroblasts in the presence or absence of the macrophage-like cell line RAW264 cells revealed that LPS stimulation of iNOS was inhibited by nifedipine and could mediate the nifedipine inhibition of apoptosis (Fujimori et al. 2001). Whether this effect of nifedipine occurs in vivo or with primary human macrophages has yet to be determined, but could be a contributing mechanism of the inhibited apoptosis in nifedipine-induced gingival overgrowth observed in human gingiva (Kantarci et al. 2007).

Molecular Aspects of Fibrotic Forms of Gingival Overgrowth

The expression and regulation of CCN2 in gingival tissues and cells have been informative with respect to possible mechanisms of tissue specificity of fibrotic forms of gingival overgrowth. CCN2 is a reliable marker of fibrosis and contributes

Figure 2. Summary of signal transduction relationships in human gingival fibroblasts compared with lung fibroblasts. Unique aspects are indicated. ALK5, EP2, and EP3 are receptors that mediate signaling by TGF- β and prostaglandin E₂ (PGE₂); * high in gingival fibroblasts only; || high in lung fibroblasts only (Black et al. 2007; Thompson et al. 2010).

to fibrosis development initiated by TGF- β (Mori et al. 1999). Major drivers of phenytoin-induced gingival overgrowth appear to include TGF- β -driven CCN2 expression. At present, the cellular sources of increased TGF- β in gingival overgrowth tissues are unclear, but it is likely that higher TGF- β levels result from perturbed interactions between factors elaborated by inflammatory cells and gingival mesenchymal cells. Interference of TGF- β induction of CCN2 is, therefore, an attractive potential therapeutic target to attenuate fibrotic gingival overgrowth development. These studies are most relevant to phenytoin- and possibly nifedipine-induced gingival overgrowth and idiopathic human gingival overgrowth, which are fibrotic, unlike ciclosporin-induced lesions (Uzel et al. 2001). CCN2 is strongly and rapidly up-regulated by TGF- β in a variety of cells derived from multiple tissues, including human gingival fibroblasts and epithelial cells (Kantarci et al. 2006). Gingival fibroblasts, however, are unusual in that PGE₂ only slightly down-regulates CCN2 levels, in sharp contrast to lung and kidney fibroblasts, in which CCN2 is dramatically down-regulated by PGE₂ (Ricupero et al. 1999; Black et al. 2007). These findings led to the hypothesis that fibroblasts in gingival tissues have altered signal transduction relationships that confer unexpected resistance to effects of some inflammatory mediators which could contribute to the tissue specificity and fibrosis which occur in phenytoin-induced gingival overgrowth. Indeed, the activation of the EP3 receptor which occurs in gingival fibroblasts and not in lung or kidney fibroblasts was determined to enhance TGF- β 1 stimulation of JNK MAP kinase activation and thereby to enhance CCN2 elevation in gingival cells. In addition, the cAMP response to PGE₂ was found to be weak and delayed in gingival fibroblasts (Black et al. 2007). Subsequent studies of signaling in gingival and lung cells identified CDC42 and RAC1 but not RHOA as mediators of TGF- β 1 up-regulation of CCN2 (Black and Trackman 2008) (see Fig. 2 for a summary). Moreover, an unexpected inhibition of TGF- β 1-stimulated CCN2 levels by the canonical WNT signaling pathway, and by inhibition of glycogen synthase kinase 3-beta (GSK3 β) activity, was uncovered, which is opposite that which occurs in other biological systems (Bahammam et al. 2013). Relative levels of PKA and

Figure 3. Model for relationships between active protein kinase A and GSK3 β . The proposed model may account for the unique sensitivity of (A) human gingival fibroblast expression of TGF- β -stimulated CCN2 to GSK3 β inhibitors compared with (B) lung fibroblasts (Bahammam et al. 2013).

GSK-3 β expression have been postulated to differ between gingival fibroblasts and lung cells based on sequential and repressing and activating phosphorylations of CREB by PKA and GSK-3 β , respectively. The model summarized in Figure 3 posits that gingival fibroblasts have an unusually low ratio of activated PKA to GSK3 β . The observed low cAMP response of gingival fibroblasts to PGE₂ is consistent with this hypothesis because PKA mediates many cellular responses to cAMP (Black et al. 2007). A low level of PKA-dependent phosphorylation of CREB at Ser133 in gingival fibroblasts is predicted to repress CCN2 expression because non-phosphorylated CREB and initially phosphorylated CREB on Ser133 would fail to activate and repress, respectively, CCN2 expression (Bahammam et al. 2013). A subsequent GSK-3 β -dependent phosphorylation of P-Ser133-CREB on Ser129 would relieve this repression (see Fig. 3A). Therefore, inhibition of GSK-3 β would attenuate CCN2 expression as was observed (Bahammam et al. 2013). By contrast, lung fibroblasts would be expected to have

a high level of PKA, consistent with the observed high cAMP response to PGE₂ (Black et al. 2007). The relatively deficient low GSK-3 β level was proposed to only marginally relieve the repression of CREB robustly phosphorylated on CREB SER133 by PKA (Fig. 3B). Thus, GSK-3 β inhibitors would have little ability to further repress CCN2 in lung fibroblasts, also as observed (Bahammam et al. 2013). This hypothesis provides a model for further investigation of the apparent tissue-specific interactions between TGF- β and GSK3 β signaling (Bahammam et al. 2013).

An additional insight into CCN2 regulation in gingival fibroblasts is that several parallel pathways work together to up-regulate CCN2 in gingival fibroblasts. Targeting any one of these pathways reduces CCN2 expression, and presumably also gingival overgrowth. Agents that target different arms of these parallel pathways used together are more effective than each agent alone, due to the additive effects of targeting these parallel non-redundant regulatory signaling relationships. One example is the combination of lovastatin plus forskolin. Lovastatin inhibits the activation of the small G-proteins RAC1 and CDC42, which contribute to TGF- β -stimulated CCN2 expression (Black and Trackman 2008). In addition, lovastatin has been reported to inhibit GSK-3 β activation, which could result in elevated repression of CCN2 as proposed in Figure 3 (Lee et al. 2012). Forskolin increases cAMP levels by directly activating adenylate cyclase and thereby activates protein kinase A. This leads to increased levels of CREB phosphorylated on Ser133, but perhaps not to a sufficient level for subsequent Ser129 phosphorylation by GSK3 β , especially in the presence of lovastatin, which inhibits GSK3- β activity. The predicted outcome is strong repression of CCN2 (Fig. 3). Indeed, data analysis showed that the combination of lovastatin and forskolin inhibits CCN2 levels in an additive manner (Black et al. 2007; Black and Trackman 2008). It is clear that additional detailed mechanistic studies are needed to confirm and extend this understanding to utilize these findings to develop new clinical strategies to address gingival overgrowth.

Clinical Implications

Findings summarized above regarding tissue-specific responses of human gingival fibroblasts have clear clinical implications. The hypothesis was developed that statins would have the potential to prevent or treat gingival overgrowth. This is because statins inhibit HMGCoA reductase (Betteridge et al. 1978), which is required for the biosynthesis of mevalonate-derived active geranylgeranylated RHO family small G-proteins, including RAC1 CDC42, and RHOA [reviewed in Vallianou et al. (2014)]. As noted above, active RAC1 and CDC42 are uniquely required for TGF- β -stimulated CCN2 levels in gingival fibroblasts [Fig. 2; Black and Trackman (2008)]. Statins are widely prescribed for treating hypercholesterolemia and, as approved drugs, are of particular interest as a potential therapeutic option for addressing gingival overgrowth. Preliminary studies in a mouse model of gingival overgrowth suggest that a

statin drug can prevent phenytoin-induced gingival overgrowth in vivo (Assaggaf, Kantarci, Sume, and Trackman, manuscript submitted). Similarly, local application to gingiva of pharmacologic GSK-3 β inhibitors in in vivo models of gingival overgrowth could also be of interest. To our knowledge, no human clinical studies investigating the ability of statins to prevent or treat drug-induced gingival overgrowth have been undertaken.

Gingival Overgrowth and Cancer

Clinically, gingival overgrowth and oral cancers (e.g., squamous cell oral cancer) are clearly different. However, fibrosis in and around cancer lesions is now understood to be a major component of the oncogenesis which promotes migration and intravasation of tumor cells and the formation of distant metastases (Lu et al. 2012). Another potential link between fibrosis and cancer can be seen in the example of Neurofibromatosis Type I, also known as Recklinghausen's disease, which commonly leads to gingival and tongue tissue overgrowth, which can progress to oral cancer (Cunha et al. 2004). The primary phenotype of the disease is the presence of nerve sheath tumors known as neurofibromas, which contain Schwann cells in association with mast cells, fibroblasts, and endothelial cells. This disease is caused by a variety of inactivating mutations in the *NF1* gene, which codes for neurofibromin, normally a tumor suppressor and negative modulator of RAS signaling. Approximately half of human *NF1* mutations are inherited. Intraoral lesions include neurofibromas on the tongue, lips, buccal mucosa, gingiva and papillae, floor of the mouth, alveolar ridge, and palate (D'Ambrosio et al. 1988). The NF1 protein contains multiple domains whose functions have not yet been fully elucidated. It has been determined, however, that one activity of NF1 is to inhibit RAS activity. RAS is an oncogene and a small G-protein that is active when bound to GTP and inactive when bound to GDP. Active RAS results in stimulation of pathways that lead to mesenchymal cell proliferation, including the ERK MAP kinase pathway and the AKT pathway. NF1 normally accelerates the conversion of the active GTP-bound form of RAS to the inactive GDP-bound form (Bollag et al. 1996; Le and Parada 2007). Mutant forms of NF1 are inactive and result in overactive RAS signaling, increased epithelial to mesenchymal transition, proliferation, and fibrosis (Arima et al. 2010; Widemann et al. 2014).

Similar to gingival overgrowth and oral cancer (Flynn et al. 1991; Soory and Suchak 2001; Iamaroon et al. 2003; Nurmenniemi et al. 2004; Mohtasham et al. 2010; Pyziak et al. 2013), increased levels of degranulated mast cells and macrophages are associated with neurofibromas. Analysis of initial data suggests that the macrophages in NF1 tissues are not principally synthetic macrophages (Prada et al. 2013), but additional studies are required. Macrophages at an early stage of NF1 are protective against NF1 development, while at late stages macrophages contribute to the severity of neurofibromas (Prada et al. 2013). It is currently unclear whether there is a corresponding phenotypic switch from inflammatory M1 macrophages to synthetic M2 macrophages in NF1. Interactions

between inflammatory cells and connective tissue cells leading to proliferative and fibrotic consequences are likely, but still only partially understood (Staser et al. 2010; Prada et al. 2013). Increased release of TGF- β in particular from mast cells and/or macrophages is suspected as being a driving force in the development of both gingival overgrowth and neurofibromas (Saito et al. 1996; Tipton and Dabbous 1998; Ling et al. 2005; Yang et al. 2006).

Interestingly, markers of fibrosis, including CCN2 and lysyl oxidase like-2 (LOXL2), are elevated in metastatic oral cancer (Peinado et al. 2008; Kikuchi et al. 2014). LOXL2 is a member of the lysyl oxidase family of enzymes whose activity catalyzes the final reaction required for biosynthetic collagen and elastin cross-linking. Excess lysyl oxidase enzyme activity is highly correlated with excess collagen accumulation and fibrosis (Csiszar 2001). Increased LOXL2 expression is a predictor of poor outcomes in oral cancer (Peinado et al. 2008). Preliminary findings suggest that LOXL2 levels are elevated not only in oral cancer, but also in phenytoin-induced gingival overgrowth (Assaggaf, Kantarci, Sume, and Trackman, manuscript submitted). Targeting oral fibroblast fibrotic pathways such as CCN2 regulation, which we have shown to have tissue-specific features, or LOXL2 regulation or activity may have benefits beyond approaching not only gingival overgrowth, but also oral fibrosis associated with cancers in a metastasis-permissive genetic background.

Conclusion

Drug-induced gingival overgrowth remains a significant oral health issue, particularly in the absence of sufficient attention paid to oral tissues either by physicians, dentists, or the patients themselves. This can be a consequence of lack of communication between patients and healthcare providers, or between/among the respective healthcare providers. Moreover, the prescribing of cyclosporin has been reported to be increasing (Wright et al. 2005), and phenytoin and nifedipine remain in high use. Gaining insights into the unique aspects of gingival overgrowth will ultimately lead to solutions to control this quality-of-life-limiting condition and will likely provide new insights into other oral connective tissue diseases, including oral manifestations of neurofibromatosis and possibly metastatic oral cancer.

Author Contributions

P.C. Trackman, contributed to analysis and interpretation, drafted and critically revised the manuscript; A. Kantarci, contributed to data interpretation, critically revised the manuscript. Both authors gave final approval and agree to be accountable for all aspects of the work.

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