Review article

Primary cilia and hypoxia-associated signaling in developmental odontogenic cysts in relation to autosomal dominant polycystic kidney disease – A novel insight

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ABSTRACT

Developmental cysts are pathological epithelial-lined cavities arising in various organs as a result of systemic or hereditary diseases. Molecular mechanisms involved in the formation of developmental odontogenic cysts (OCs) are not fully understood yet; the cystogenesis of renal cysts originating from the autosomal dominant polycystic kidney disease (ADPKD) has been, however, explored in much greater detail. This narrative review aimed i) to summarize molecular and cellular processes involved in the formation and growth of developmental OCs, especially dentigerous cysts (DCs) and odontogenic keratocysts (OKCs), ii) to find if there are any similarities in their cystogenesis to ADPKD cysts, and, based on that, iii) to suggest potential factors, candidate molecules, and mechanisms that could be involved in the DC formation, thus proposing further research directions. Here we suggest a possible association of developmental OCs with primary cilia disruption and with hypoxia, which have been previously linked with cyst formation in ADPKD patients. This is illustrated on the imagery of tissues from an ADPKD patient (renal cyst)

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and from developmental OCs, supporting the similarities in cell proliferation, apoptosis, and primary cilia distribution in DC/OKC/ADPKD tissues. Based on all that, we propose a novel hypothesis of OCs formation suggesting a crucial role of mutations associated with the signaling pathways of primary cilia (in particular, Sonic Hedgehog). These can lead to excessive proliferation and formation of cell agglomerates, which is followed by hypoxia-driven apoptosis in the centers of such agglomerates (controlled by molecules such as Hypoxia-inducible factor-1 alpha), leading to cavity formation and, finally, the OCs development. Based on this, we propose future perspectives in the investigation of OC pathogenesis.

1. Introduction

Cysts are epithelial-lined cavities filled with fluid, semifluid, or gaseous content. Developmental cysts arise from localized cell populations that, under certain stress conditions, alter their biological behavior. These conditions may be congenital or develop during life as a result of various hereditary or systemic disorders [1]. Sporadic types are caused by somatic mutations of key genes [2] and, in some cases, also by local non-hereditary factors (e.g. the presence of an impacted tooth) [3]. The most common types of developmental odontogenic cysts (OCs) include **dentigerous cysts** (DCs) and **odontogenic keratocysts** (OKCs). Both these types of cysts can also lead to various complications, including the loss of bone and teeth, infections, or jaw fractures [4]. DCs represent approximately 20% of all cysts in the orofacial region [5] and typically develop around the crown of an impacted tooth [3]. OKCs notably differ from other OCs in their aggressive biological behavior and high recurrence rate of 5–62% [3]. OKC could be sporadic or be a part of the nevoid basal cell carcinoma syndrome (or Gorlin-Goltz syndrome) [1,6].

Cystic diseases are, however, not limited to the orofacial cavity. Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease, characterized by the formation of multiple cysts in the kidney, or even in other organs [7]. Mutations in polycystins-coding genes Polycystic Kidney Disease 1 and 2 (*PKD1* and *PKD2*) are considered the most common causes of ADPKD [8]. These mutations lead to an aberrant functionality of a variety of interconnected signaling pathways, which results in abnormal cell proliferation, inflammation, and, subsequently, in fibrosis with accompanied cystogenesis [8].

Several candidate genes [9] and theories [3] have been proposed for the development of OKCs and DCs; however, very little is known as to the specific mechanism of their development. Even though DCs have been proven to arise from the post-functional follicular tissue (PFFT) through the accumulation of fluid between the reduced enamel epithelium and the crown of the unerupted tooth, it remains unclear how and why the transformation of PFFT to DCs occurs [3]. We believe one of the reasons for the lack of knowledge is that very little attention has been paid to the investigation of similarities in the formation of various types of developmental cysts so far. Understanding such common pathways could promote research in the field of OCs. For example, the etiopathogenesis of ADPKD cysts has been investigated in detail [10]. Primary cilia signaling [11] and hypoxia [12] have been proposed to play a crucial role in the development of ADPKD cysts, but this knowledge has never been applied in depth to the OCs research.

For these reasons, we have prepared this narrative review aiming to i) summarize the state of art in the contemporary research of DCs and OKCs related to primary cilia and hypoxia-associated pathways, ii) to identify potential similarities in cystogenesis between OCs and ADPKD cysts, and iii) to illustrate these similarities on histological imagery. Based on the above, we aim iv) to suggest potential factors, candidate molecules, and mechanisms possibly that could be involved in the DC and OKC formation, and v) to propose a novel hypothesis for DC cystogenesis. Ultimately, such a summary and synthesis of available information could facilitate and direct further research in this field that has been largely neglected for several decades.

2. The role of primary cilia and associated signaling pathways in the formation of the developmental odontogenic cysts

2.1. Primary cilia and their signaling

Primary cilia are microtubule-based, non-motile antennae-like organelles located on the surface of most mammalian cells. Under the electron microscope, they appear as projections extending from a basal body and are assembled through a process called intraflagellar transport [13].

Signaling through the primary cilia is important during odontogenesis. Physiologically, they are involved in the transduction of signals from the environment into the cell. Activation of primary cilia signaling induces specific cell responses including proliferation, migration, cellular adhesion, apoptosis, etc. [13]. Primary cilia can be observed in various types of cells in all organs, such as the surface of nephrons, the dendritic knob of the olfactory mucosa [13], or odontoblasts and ameloblasts during odontogenesis [14,15]. Defects disrupting the morphology and/or function of the primary cilia cause disorders called ciliopathies. Aberrant signaling provided by primary cilia is frequently associated with oncogenesis and cystogenesis. Primary cilia also play a role in coordinating several key signaling pathways, including the SHH (Sonic Hedgehog), Wnt (Wingless/Int-1), FGF (Fibroblast Growth Factor), or PDGF (Platelet Derived Growth Factor) pathways, as well as intracellular Ca^{2+}|cAMP signaling [13].

Many cystic diseases belong to the family of ciliopathies (e.g. nephronophthisis, Bardet-Biedl syndrome, Joubert syndrome, etc.) [13]. A few years ago, a theory has been proposed that primary cilia may also be responsible for DC development [16]. A later study that included 5 DCs, 5 OKCs, and 5 periapical cysts confirmed the presence of primary cilia in all samples of DCs and OKCs, but not in periapical cysts arising due to an infection of the root canal of the tooth [17]. This implies that primary cilia may be potentially

involved in the formation of developmental OCs, but excludes their participation in periapical cyst formation.

Members of our research team recently revealed that primary cilia are abundantly present also in the plexiform and follicular types of ameloblastoma, but only scarcely in its basal cell type form [18]. Ameloblastoma is an odontogenic tumor sharing some similarities with DCs to such a degree that distinguishing unicystic ameloblastomas associated with an impacted tooth from DCs is challenging even for pathologists [19]. In addition, several cases of ameloblastoma arising from DC [20–22] or OKC [23] were reported. Whether primary cilia and the associated pathways could play a role in the transformation of OCs into ameloblastomas, remains, however, unclear.

2.2. Polycystins

Polycystins are another group of signaling molecules that may be involved in developmental OCs formation. Polycystins, namely polycystin-1 (PC1) and polycystin-2 (PC2), are proteins that are exhibited on the membranes of primary cilia. They are encoded by the *PKD1* and *PKD2* genes located on chromosomes 16p13.3 and 4q22, respectively. Mutations of these genes are responsible for ciliopathies of nephrons and the development of ADPKD. PC2 is a member of the transient receptor potential (TRP) ion channel family whereas PC1, a transmembrane helix protein, exhibits characteristics of both ion channel protein and adhesion G-protein coupled receptor (aGPCR) [24]. In ADPKD, both the absence of PC1 and the overexpression of *PKD2* were shown to promote cystogenesis. In fact, results of studies in *PKD2* transgenic mice lines suggest that PC2-induced extracellular signal-related kinase (ERK) activation via B-Raf signaling may play an important role in cystogenesis [25].

PC1 expression in odontoblasts is critical for proper odontogenesis in the early stages of tooth development [14]. The PC1 production is also abundant in the epithelial cells of DCs, with the protein localized mainly in the cytoplasm [26]. This expression pattern correlates with the pathological cytoplasmic expression of PC1 in ADPKD (in physiological nephrons, PC1 acts as a transmembrane protein) [27]. Therefore, it is likely that mutations in *PKD1* or its pathological expression may also participate in the formation of DCs. Nadar Singarayan et al. also suggested that the PFFT tissue may transform into a DC over time due to the increased proliferation of a monoclonal PFFT cell with mutated *PKD1*, forming favorable circumstances for cystogenesis [26].

The most recent study published on polycystin expression confirmed the presence of PC1 not only in DCs, but also in OKCs and ameloblastomas; however, differences in the proportion of positive samples as well as in the intensity of expression were found among these tissues [28]. Immunohistochemistry showed PC1 expression in 22 out of 29 ameloblastomas, 5 out of 17 OKC samples, and only 3 out of 20 DCs, respectively. In addition to PC1, Li et al. also investigated PC2 expression in the same samples, revealing positive results in 25 out of 29 ameloblastomas, 7 out of 17 OKCs, and 9 out of 20 DCs, respectively. Gingival tissues did not express PC1 nor PC2 [28].

Another important aspect of PC1, besides its function in mechanotransduction, lies in its contribution to intercellular contacts and cell-matrix interactions. In these contacts, PC1 possibly modulates the junctional turnover and influences tissue organization [28,29]. PC1 also exhibits the ability to interact with the extracellular matrix (ECM) through intermediate filaments. The co-localization of PC1 with $\alpha_2\beta_1$ integrins supports its role in the ECM-cell interplay [30]. Moreover, integrins are known to act as receptor proteins binding to ECM. Thus, if any changes in the ECM composition occur, integrins function as sensors triggering cell response to such changes in the sense of cell attachment rearrangement, alterations of migration, differentiation, and proliferation. Cells of the epithelial lining in DCs were demonstrated to overexpress integrin-α_2 and integrin-β_1, which, in turn, caused increased cell adhesion [31]. The upregulated expression of adhesion molecules in DCs might result from the increased pressure acting on the epithelial lining due to continuous fluid absorption. The enlarging cyst demands increased intercellular contacts and a higher adhesion rate to maintain the epithelial lining. This would explain the higher pathological cytoplasmic expression of PC1 instead of its membranous exhibition in DC [31].

Similarly, $\alpha_2\beta_1$ integrins are also overexpressed in ADPKD cysts where the α_2 subunit is associated with the cell stratification of the epithelial lining and the β_1 subunit participates in the flow-induced Ca^{2+} response in the cilium of the kidney. An enhanced expression of both integrins was also linked to the resistance to anoikis in PC1 knockdown cells [32]. However, inactivation of the β_1 subunit in PC1-deficient animal models radically inhibited kidney cyst formation [33]. Besides integrins, ECM and focal adhesion signaling are considered to play a crucial role in ADPKD development [34].

2.3. Sonic Hedgehog signaling

Sonic Hedgehog signaling (SHH) involves multiple molecular and cellular functions essential in organogenesis, cell proliferation, and differentiation during the embryonic period including tooth and craniofacial development [1].

The alterations of the Patched receptor (PTCH) homolog 1 due to the loss of heterozygosity and the subsequent activation of SHH have been proposed as a probable causative factor initiating DC cystogenesis [2,6]. Genetic variations in *PTCH1* have also been described in syndromic and non-syndromic OKCs [1,9]. Immunohistochemical examinations of various OCs (including DCs and OKCs) and odontogenic tumors (OTs) revealed positive staining for SHH-related proteins such as Smoothened (SMO), PTCH, or glioma-associated oncogene homolog 1 (GLI1) [35]. *PTCH1* and *GLI2* were found to be highly expressed in human primary OKCs [36] as well as in renal cysts obtained from *PKD1* patients [37]. Despite this universal expression of PTCH in all examined lesions [35], it is likely that the different mutations in the *PTCH1* would affect expression in a lesion-specific way [1]. In addition, SHH signaling can also possibly influence related pathways. Mutations affecting both alleles of *PTCH1* are very common in sporadic OKCs [38,39]. The distribution of various kinds of *PTCH1* mutations was found to differ between sporadic and syndromic OKCs [40].

PTCH-positive immunostaining can be detected even if the protein is truncated due to the mutation in the encoding gene and as long as at least one allele for PTCH is functional, cells do not change their biological behavior [1]. However, the occurrence of an additional stimulus causing a mutation in the other allele leads to the increased proliferation of the progenitor cells, which further

spreads out throughout the whole reduced enamel epithelium and transforms the follicular epithelium into a DC. Nevertheless, other interactions may also be involved in this cystic transformation, representing challenges for future research.

Besides the tumor suppressor *PTCH1* mutation in both hereditary and sporadic OKCs, mutations in the *SMO* gene were also described a few years ago in patients with these cysts, indicating the possible contribution of *SMO* mutations to OKC development [41, 42]. Hoyos Cadavid et al. pointed out that syndromic OKCs harbor SHH proteins (including SMO and GLI1) significantly more than sporadic OKCs [43]. Still, as no *SMO* mutations were found in several studies, this type of mutation seems to be rather rare in OKCs [44, 45]. On the other hand, as Rodriquez et al. pointed out, high *GLI1* expression is commonly present in OKCs and the degree of its expression seems to play a significant role in the aggressiveness of this type of cyst [46]. In line with this, Ohki et al. reported a higher *GLI1* expression in syndromic OKCs than in sporadic ones [47], which might explain the more aggressive nature of OKCs in Gorlin-Goltz syndrome compared to sporadic types [43].

Similarly, the SHH signaling pathway may contribute to ADPKD development as well. Increased expression of SHH components was demonstrated in renal ADPKD tissue [37] and downregulation of SHH signaling was proven to be able to arrest the formation of these cysts [48]. Further, inhibitors of the SHH signaling, including SMO and GLI1 (inhibitors SANT2 and GANT61, respectively), significantly reduced cell proliferation in ADPKD cells [49]. Thus, SHH signaling was proposed to be necessary for the cystic transformation of renal tissue [49]. On the other hand, cell-autonomous SHH signaling has not been recognized to participate in cyst development in ADPKD murine models with *PKD1* inactivation, and the loss of *PKD1* function in cystic kidney disease was related to signaling pathways other than SHH [50]. Therefore, the role of the SHH pathway in ADPKD remains largely unexplained and further investigation is needed for its full clarification.

3. The role of HIF-1α, Notch, and caspase-3 in the odontogenic cysts and ADPKD cyst formation

Increased epithelial proliferation during odontogenic cyst development results in the formation of epithelial islands. When these epithelial clusters grow, the amount of nutrients and oxygen supplied to the cells in the central area of the agglomerate is limited, which inevitably leads to cell necrosis/apoptosis. This was previously proposed as a notable mechanism taking place during odontogenic cyst formation [51,52]. This process results in the development of a cavity with a hyperosmolar environment formed by the abundance of intracellular content resulting from cellular breakdown. The hyperosmolality further attracts liquid from the surrounding tissue, causing the cyst to expand [3].

The Hypoxia-inducible factor-1 alpha (HIF-1α), one of the proteins associated with hypoxic conditions, is known to affect cell proliferation, angiogenesis, and apoptosis, i.e., multiple processes contributing to cystogenesis [53]. HIF-1 also affects the expression of vascular endothelial growth factor (VEGF), which is necessary for angiogenesis, and the inducible nitric oxide synthase (iNOS), generating nitric oxide responsible for vasodilation and increased blood flow to ischemic tissues [54,55].

Overexpression of HIF-1α plays a key role in autophagy and cell apoptosis after initiating the caspase-dependent cascade of events [56]. Caspase-3 was highly expressed in OCs compared to OTs; moreover, the protein was present both in the nucleus and the cytoplasm while in OTs, it was predominantly cytoplasmic. This strongly indicates DNA fragmentation and supports the hypothesis that apoptosis plays an important role in the DC development [51].

Hypoxia-related events have been associated with cyst formation in ameloblastoma as well [57–59]. Immunochemical investigation of the expression of HIF-1α and caspase-3 in cystogenesis of various OCs, OTs, and PFFT revealed their presence in all cysts and tumors [51]. However, the intensity, cellular localization, and epithelial layer distribution of the expression of these caspases varied for individual types of lesions. HIF-1α was found to be highly expressed in DCs and its expression was comparatively higher in radicular cysts than in odontogenic tumors [51]. High expression of HIF-1α in these OCs when compared to gingival tissue was also recently confirmed [60]. Interestingly, HIF-1α was detected in cystic regions of ameloblastomas both in the nuclei and the cytoplasm, while solid regions of ameloblastomas expressed HIF-1α only in the nuclei [61]. This indicates that in cystic regions, HIF-1α is stabilized in the cytoplasm. Normally, HIF-1α normally enters the nucleus under hypoxic circumstances and there, it binds to its beta subunit and triggers events leading to the adaptation to hypoxia, thus improving the resistance to apoptosis [58,61]. However, if HIF-1α remains in the cytoplasm and only reduced amounts reach the nucleus, adaptation does not take place and the cells enter apoptosis, which may lead to the formation of a cystic cavity [61].

HIF-1α was upregulated both in DCs (compared with the dental follicular tissue) [51] and OKCs (compared with healthy oral mucosa) [62,63]. This could play an important role in establishing membrane projections with the ability to decompose ECM, called invadopodia, hence aiding the expansion of the cyst [62]. Further, elevated expression of other molecules, such as Notch1, was discovered in OKCs (compared to the healthy oral mucosa and other OCs such as the calcifying odontogenic or orthokeratinized odontogenic cyst). Both pathways are believed to contribute to the aggressive behavior of tumors and both are regulated by HIF-1α [62]. The overactivation of Notch1 in OKCs and DCs, compared to inflammatory OCs (radicular cysts), suggests its important role in the pathogenesis of these developmental OCs [64]. Moreover, the inhibition of the SHH pathway by an SMO antagonist cyclopamine was correlated with the downregulation of the Notch pathway as well as with the growth arrest of OKC cells [36]. A recent study using the OncoScan assay revealed a loss of heterozygosity in the *Notch1* gene in both syndromic and sporadic OKCs [65].

This HIF-1α role in OCs represents another possible link to ADPKD, in which HIF-1α was also rather associated with cyst progression than with the initiation of cystogenesis [12]. Its expression is stage-dependent, and the reduced local tissue oxygenation is caused by the compression of the adjacent nephrons and peritubular vasculature during the cyst growth [66]. Several studies verified the causative link between caspase-3 and ADPKD [67–69]. The mitigating effect of caspase-3 inhibition (using a pan-caspase inhibitor) was observed in rat models of ADPKD. It was further revealed that the inhibition of caspase-3 not only reduced apoptosis but also decreased tubular cell proliferation, which is a prerequisite for cyst expansion [68]. The activation of caspase-3 and dysregulation of

Table 1

Comparison of selected factors involved in cystogenesis in dentigerous cysts (DCs), odontogenic keratocysts (OKCs), and autosomal dominant polycystic kidney disease (ADPKD) cysts.

Molecules/Pathways	Findings in DCs	Reference	Findings in OKCs	Reference	Findings in ADPKD cysts	Reference
SHH	Mutation in *PTCH1* plays a role in the initiation of cyst development	[6] [2] [9]	Mutation in *PTCH1* plays a role in the initiation of cyst development	[1] [9] [65]	Upregulation of SHH-related genes (*PTCH1*, and *GLI2*) assessed in ADPKD human tissue samples by performing cDNA microarrays Downregulation of SHH reduces cystogenesis in mouse models Upregulated *GLI1*, in human ADPKD renal tissue, inhibitors of SHH reduce cyst formation In *PKD1* conditional mouse mutants, SHH is not involved in cystogenesis	[37] [48] [49] [50]
Notch signaling	Notch1 overexpression observed by immunostaining is possibly involved in growth and expansion of the cyst	[64]	Notch1 overexpression observed by immunostaining is possibly involved in growth and expansion of the cyst	[64] [62]	Inhibition of Notch3 decreased cell proliferation and cyst formation and growth in human ADPKD cells Inhibition of Notch decreased cell proliferation and cyst growth in human ADPKD cells	[70] [71]
HIF-1α	Overexpression observed by immunostaining, involved in apoptosis, cyst formation and lesion growth	[51] [60]	Overexpression observed by immunostaining, involved in apoptosis, cyst formation and lesion growth	[51] [62] [63] [60]	Overexpressed in ADPKD rat and mouse model; could be involved in cyst expansion Involved in ADPKD progression and cyst expansion	[53] [12]
Caspase-3	Mostly moderate to strong expression by immunostaining Positive immunostaining Overexpression observed by immunostaining, involved in cyst formation and lesion growth	[54] [72] [51]	Mostly marginally positive or broadly positive expression observed by immunostaining Mostly moderate to strong expression observed by immunostaining Uncommon expression by immunostaining Overexpression observed by immunostaining, involvement in lesion growth Uncommon expression by immunostaining	[73] [54] [74] [51] [75]	Overexpression observed by use of fluorescent substrates in an ADPKD rat model suggesting an important role in cyst formation Caspase-3 inhibition slows cyst progression in a polycystic kidney disease rat model *Caspase-3* gene deletion prolongs survival in congenital polycystic kidney mouse models	[67] [68] [69]
PC1, PC2	Positive cytoplasmic immunostaining of PC1 suggesting a role in transformation of PFFT do DC Positive cytoplasmic and membranous immunostaining of PC1 and PC2	[26] [28]	Positive cytoplasmic and membranous immunostaining of PC1 and PC2	[28]	Upregulation of cytoplasmic PC1 observed in human ADPKD tissue samples by performing immunohistochemistry Mutations in *PKD1* and *PKD2*, which encode PC1 and PC2, belong to the most common causes of ADPKD	[27] [8]
α2β1 integrins	Positive immunostaining with diffuse distribution in 14 out of 21 specimens. Involved in squamous metaplasia of reduced enamel epithelium to stratified epithelium	[31]	Overexpression of the α2 subunit observed by immunostaining, the thickness of the epithelial lining correlated with the expression of integrin molecules	[76]	Upregulation of α2β1 integrins in cell cultures with knockdown of PC1 results in resistance to anoikis, suggesting a role in cyst expansion Inactivation of β1 integrin suppresses cyst growth in ADPKD models	[32] [77]

DCs – dentigerous cysts, OKCs – odontogenic keratocysts, ADPKD – autosomal dominant polycystic kidney disease, SHH - Sonic Hedgehog pathway, PC1 - Polycystine 1, PC2 – Polycystine 2, *PKD1* - polycystins-coding genes Polycystic Kidney Disease 1, *PKD2* - polycystins-coding genes Polycystic Kidney Disease 2, HIF-1α - Hypoxia-Inducible Factor-1 Alpha, *PTCH1* - Patched receptor homolog 1 gene, *GLI1* – Glioma-associated homolog 1 gene, *GLI2* – Glioma-associated homolog 2 gene, α2β1 integrins – alpha two and beta one integrins.

*The results of the studies on SHH in ADPKD are not entirely consistent, since the role of SHH was not confirmed in *PKD1* conditional mouse models [50]. Therefore, the role of SHH in ADPKD is not clear.

the balance between the pro- and antiapoptotic B-cell lymphoma 2 (Bcl-2) family members correlates with increased apoptosis in ADPKD rat models [67].

Similar to OKCs, upregulated Notch signaling was found to be important for cyst formation and growth in ADPKD [70]. According to a recent study, Quinomycin A not only inhibited Notch signaling; at the same time, it targeted HIF-1α, strongly suggesting the crosstalk between HIF-1α and Notch signaling [71]. It is also speculated that the inhibition of Notch signaling shortens primary cilia, which may result in better patient prognosis as elongated primary cilia were associated with ADPKD [70]. All this suggests an important role of HIF-1α and Notch in cyst enlargement.

4. Comparison of selected factors involved in cystogenesis in dentigerous cysts, odontogenic keratocysts, and ADPKD cysts

The above-presented overview of studies investigating cellular mechanisms and signaling pathways (SHH, Notch, HIF-1α, caspase-3, PC1, α2β1 integrins) involved in the development DCs and OKCs reveals a number of similarities with ADPKD (Table 1).

Moreover, to illustrate the possible links between DCs, OKCs, and ADPKD, we performed immunohistological staining and analyzed the microscopic anatomy in cyst samples from patients with DC, OKC, and ADPKD (Fig. 1; patients' histories and methods used are presented in Supplement 1). We found some similarities in the distribution and amount of proliferating cells, apoptosis, and primary cilia distribution in these lesions. While only sparse primary cilia were observed in the basal layer of the epithelial lining of the DC, the
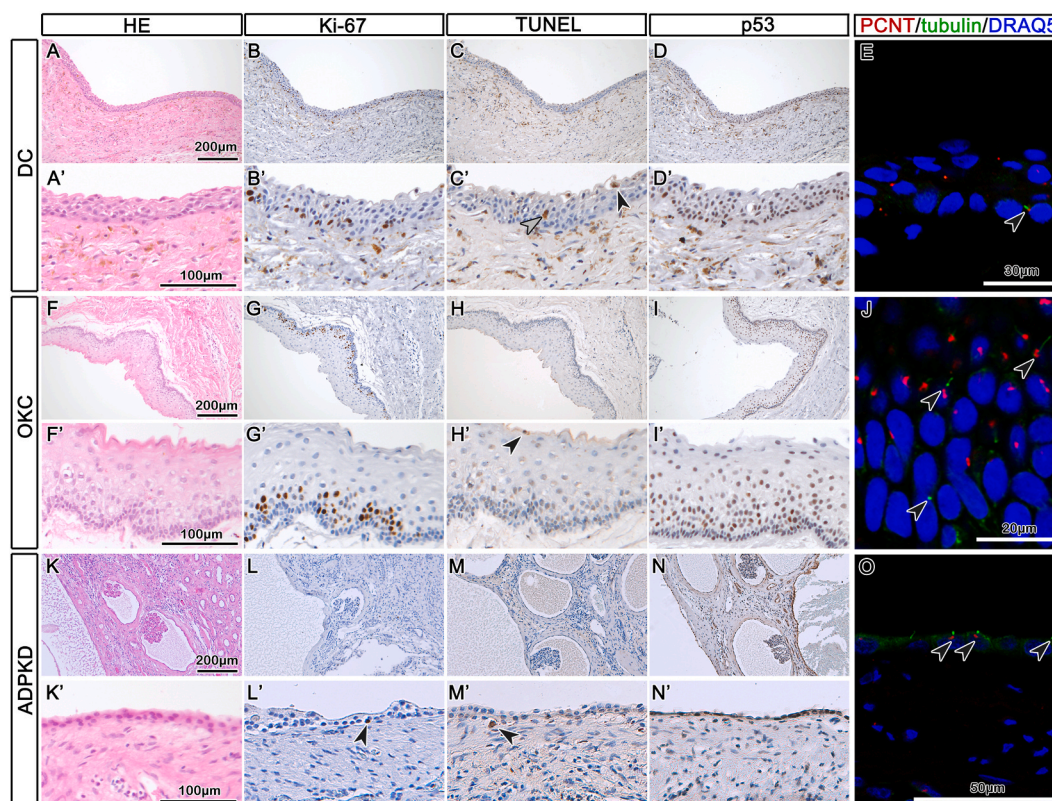

Fig. 1. Microscopic anatomy of dentigerous cyst (DC), odontogenic keratocyst (OKC), and autosomal dominant polycystic kidney disease (ADPKD): (A, A′) The wall of DC consists of a thin epithelial layer and dense connective tissue with a variable number of fibroblasts. Pigment deposition (hemosiderin) or cholesterol crystals are located in the connective tissue. The epithelial lining consists of non-keratinized stratified epithelium with a moderate degree of cellular dystrophy. (B, B′) DCs exhibit low proliferation activity mainly in the basal layer of the epithelium. (C, C′) The apoptotic rate (TUNEL-positive cells) is low, and rare apoptotic bodies (black arrowhead) are dispersed in the epithelium (pigment deposition – grey arrowhead). (D, D′) Epithelial lining of DC exhibits a moderate expression of p53. (E) There are only sparse primary cilia in the basal layer of the epithelial lining of the DC (arrowhead). (F, F′) The epithelial component of OKC is more prominent compared to the DC, the epithelium is stratified and keratinized with parakeratosis and intracellular edema. (G, G′) OKC exhibits mitotic activity similar to DC, with prominent nests of proliferating cells. (H, H′) The apoptotic rate of OKC is low, with only occasional apoptotic bodies located in the outer layers of the epithelium (arrowhead). (I, I′) Epithelial lining of OKC demonstrates moderate expression of p53. (J) The OKC displays large numbers of primary cilia in the basal layer of epithelium, with random orientation and different lengths (arrowheads). (K, K′) Glomerular cysts and large cysts lined by simple cuboidal epithelium are typical features of ADPKD. (L, L′) The epithelium lining of cysts in ADPKD displays very low mitotic activity with only occasional Ki-67 positive cells (arrowhead) and the apoptotic activity is similarly low (arrowhead) (M, M′). (N, N′) The epithelium of tubules and the epithelial lining of cysts are positive for p53. (O) Short primary cilia are present on the cells of the flattened epithelial lining of cysts in ADPKD (arrowheads).
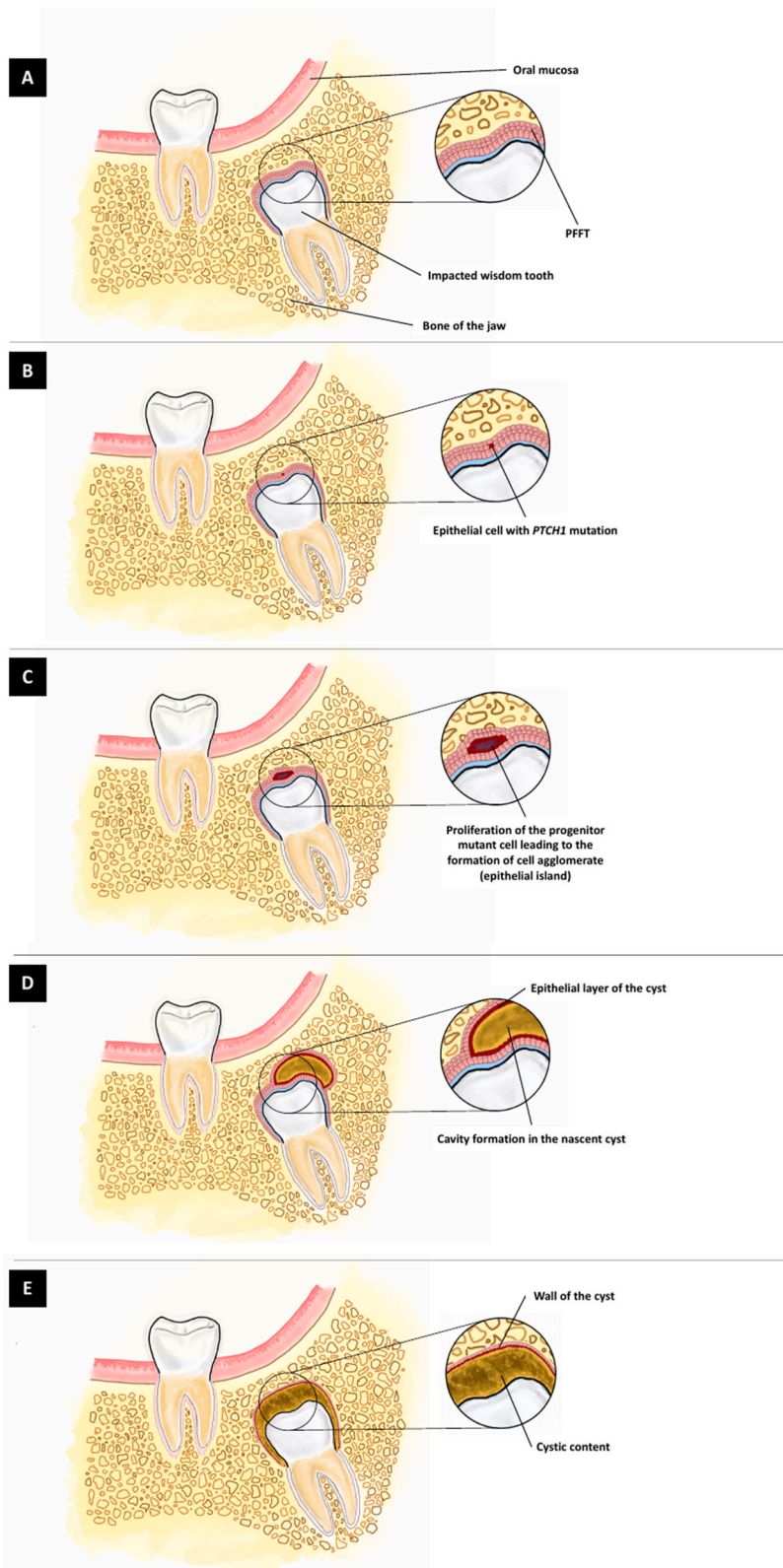
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Fig. 2. Hypothesis for dentigerous cyst (DC) formation: (A) Developed permanent retained tooth (the third molar) in the mandible. (B) Due to a specific mutation, such as the loss of heterozygosity caused by a second hit in the encoding gene for Patched receptor homolog 1 (*PTCH1*), the Sonic Hedgehog pathway in the primary cilia is over-activated, causing a monoclonal proliferation of the mutated cell (red color) in the stellate reticular layer of enamel organ [2,6,9]. (C) In the specific microenvironment, local hypoxia develops in the center of the monoclonal cell cluster (purple color). Hypoxia activates the expression of the hypoxia-inducible factor-1 alpha (HIF-1α), which leads at the same time to apoptosis and prolif-eration [51]. (D) The proliferation is dominant on the margins, while the cells of the reduced enamel epithelium in the center cease to proliferate; in this way, a cavity is formed. The caspase-3, as a promotor of apoptosis, is overexpressed [51,54]. The hyperosmolar environment created by the accumulation of cell breakdown products absorbs fluids from the vicinity (green-brown color) and exerts pressure on the epithelial lining [3]. The epithelial cells produce cytoplasmic polycystin (PC1), which upregulates the expression of $\alpha_2\beta_1$ integrins, thus increasing the cell adhesive rate [26, 31]. The cyst is lateral to the crown of the tooth (the crown may be entirely included in the cyst sac), while the root is outside the cystic sac. (E) The cyst is attached to the tooth neck at the cement-enamel border, which limits its further growth in this direction but it can continue to grow into the bone. In this phase, enamel epithelial cells, which are in contact with the crown of the tooth, die because they are under pressure and have no nutrition (hypoxia). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

OKC displays large numbers of primary cilia in the basal layer of the epithelium, with random orientation and different lengths; short primary cilia are present on the cells of the flattened epithelial lining of cysts in ADPKD. The epithelial lining of DC and OKC samples exhibited moderate expression of p53, while the epithelium of tubules and the epithelial lining of renal cysts from a patient with ADPKD were highly positive for p53. These differences in p53 expression might be one of the factors causing the differences in the clinical behavior of individual types of cysts.

5. A proposed hypothesis for dentigerous cyst formation

Based on the above, we hypothesize that the process of DC formation is initiated by a "driver mutation" in a cell of the PFFT (Fig. 2). We consider the loss of heterozygosity caused by one or more mutations in the *PTCH1* to be a probable etiological agent of DC development [2,6,9]. As a result, SMO is released and the SHH pathway is activated in a ligand-independent manner. SHH most likely influences also other signaling molecules and genes, such as p53 or *PKD1* [9,26]. The interplay between the pathways bolsters the proliferation of the progenitor mutant cell, which results in the transformation of PFFT into a DC. The increased proliferation in the center of the cell cluster causes oxygen depletion, creating a hypoxic environment. This promotes hypoxia-inducible factors [51,54, 64], which are responsible for the stimulation of apoptosis and proliferation. The central part of the tissue breaks down due to cell apoptosis (thus creating a cavity) while cells at the margins continue to proliferate. The cyst enlargement process is facilitated by the fluid drawn into the cavity due to the hyperosmolar environment formed by the products of cell breakdown. The hydrostatic pressure inside the cavity acts on the epithelial cells of the cystic wall. Subsequently, mechanoreceptors on the primary cilia transduce the signal into the cell, which leads to a pathological cytoplasmic PC1 overexpression [26]. This upregulates the production of integrins on the membrane [31], strengthening the epithelial lining through higher cell adherence to endure hydrostatic pressure. Although Notch1 overexpression was found in OCs [64], the precise role of this signaling pathway in the process of DC formation remains unclear.

The process of OKC development is also likely to be analogical to that we propose for DCs in Fig. 1.; nevertheless, mutations and upregulation/downregulation of other genes than *PTCH1* may contribute to its more aggressive nature, especially in the syndromic ones (e.g. *Notch1*, as mentioned above) [9,65]. Looking at the development of ADPKD cysts, however, we can see that polycystins including PC1 are crucially involved in renal cyst development and mutations in their genes are the main etiological factor [78]. Considering the number of analogies on the molecular and cellular levels, we can see that the main difference in the development of the discussed jaw cysts vs ADPKD is the causative factor (mutation in *PTCH1* vs *PKD1*).

6. Future perspectives and clinical implications

Primary cilia, a cellular structure crucially involved in ADPKD development, seem to also contribute to many diseases and con-ditions of the craniofacial area. Still, the precise function of primary cilia of dental cells and signaling pathways that pertain to this cellular structure are still poorly understood and require more attention in research, including the research on OCs [79,80]. While Moore proposes several directions for further investigation of primary cilia in craniofacial research in general, he does not elaborate on its role in OC development [79].

Besides, emerging evidence suggests that *PKD1/PKD2* mutations are responsible not only for ADPKD, but also for some craniofacial anomalies in a mouse model and for specific facial characteristics in humans [81]. Hypoxia is another important driver of ADPKD progression [12]; it has, however, only recently drawn attention in the research of OC pathogenesis [51].

In addition to the signaling pathways presented in the proposed mechanism (Fig. 2), additional signaling pathways may be involved in the formation of OCs. Several signaling molecules, such as p53 [9], VEGF [54], or iNOS [55], were shown to be significantly overexpressed or underexpressed in these lesions. It is important to note that hypoxia precedes both p53 expression and angiogenesis, and HIF-1α directs p53 [61] and VEGF activation [58]. Although this does not necessarily mean that these molecules are directly involved in the formation of the cyst (as the change in their expression might rather reflect subsequent metabolic alterations occurring in the OCs), their possible role in OC formation should still be investigated in greater depth.

Further experiments investigating the analogies between signaling pathways leading to ADPKD and OCs could shed more light on the development of OCs. Experiments with renal subcapsular transplantation of tooth germs in mice revealed differences in the his-togenesis of DCs and OKCs. While the cavity of OKC-like lesions developed from the enamel epithelium at the early stage after

transplantation in that experiment, the cavity of DC-like lesions was formed at a later stage, resulting from cystic degeneration of the stellate reticulum of the enamel organ [82].

A wide variety of cellular and animal models have been developed to investigate the etiopathogenetic mechanisms and related signaling pathways underlying the initiation of ADPKD [10]. So far, however, no animal model has been created for the investigation of DCs. The development of OKCs was, nevertheless, described in specific models in mice with the Gorlin-Goltz syndrome phenotype [83]. A confirmation of the role of the above-mentioned mechanisms could subsequently open the door for further research. For example, experiments with transgenic mice with ablated cilia could shed more light on the importance of primary cilia during DCs formation [84].

Such investigations can also open the possibility of implementation of non-surgical treatment in developmental OCs, similar to treatment methods currently considered in ADPKD. Fenoldopam, an antihypertensive agent, impacts the length of primary cilia of osteocytes and promotes bone formation by enhancing their mechanotransduction function [79]. This drug might be a perfect candidate for the investigation of possible therapeutic effects on lesions arising in the jaws, including OCs. SHH inhibitors, such as cyclopamine [36,85] or kinase inhibitors, including sunitinib and erlotinib [86] can be considered for their treatment. Visomodegib was already shown to be effective in treating patients with syndromic OKCs, leading to a nearly complete remission [87,88]. The positive effect of vismodegib was also recently proved on cellular models simulating OKCs [89]. On the other hand, vismodegib was not always as effective as anticipated in a clinical setting and the treatment can be accompanied by adverse effects, such as nausea or dysgeusia. It is also speculated that in some cases, it might aggravate the Gorlin-Goltz syndrome [90]. Sonidegib, another SHH inhibitor, might be another potential candidate for the treatment of OCs, as suggested by its effect on ameloblastoma formation [18]. Allopurinol might also deserve attention for the treatment of OCs – it was shown to interact with *PKD2* and, therefore, it may affect lesions, in the development of which *PKD2* plays a role [28].

Inhibitors of apoptosis might be possibly also used in the treatment of OCs. Inhibitors of the mammalian target of rapamycin and caspase inhibitors were already successfully tested in ADPKD mice models [91]. This could be interesting, in particular, in the treatment of OKCs as this type of cyst was shown to significantly express the mammalian target of rapamycin [92].

7. Conclusion

We propose that similar to ADPKD, molecular mechanisms associated with primary cilia and hypoxia could play a significant role in the formation of DCs and OKCs. Mechanisms that can be common to both these types of lesions are summarized in this review and lay grounds for the proposed hypothesis of the formation of these cysts. In particular, SHH seems to play a key role in cyst initiation, possibly influencing the expression of other molecules, such as polycystins. The rapid proliferation of thus affected cells leads to the formation of cell agglomerates with hypoxic centers. Hypoxia can then lead to a further proliferation on the periphery of the agglomerate, while apoptosis is triggered in its center, which contributes to cavity formation and enlargement. Based on the above, new perspectives and potential methods for non-surgical treatment are proposed for future research. Undoubtedly, the hypotheses resulting from our review and analysis of the current knowledge need further investigation, ideally using an animal model with DC phenotype. However, if future studies confirm the importance of the above-described mechanisms, the knowledge and hypothesis summarized in this review may pave the way to new treatment opportunities.

Ethics approval

The study was approved by the Ethics Committee of the Faculty of Medicine, Masaryk University Brno, Czech Republic (No. 7/2020, March 11th, 2020) and by the Ethics Committee of the University Hospital Brno, Czech Republic (No. 08-120619/EK, June 12th, 2019). The informed consent was obtained from all participants, prior to their inclusion in the study and sample collection, in line with the Helsinki Declaration. At least three samples were analyzed for each condition.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17130.

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