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Review

Immunohistochemical patterns in different stromal variants of pleomorphic adenomas: literature review

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Summary. Pleomorphic adenoma is the most frequent type of benign salivary neoplasm located in the mouth and is characterized by its significant histopathological diversity. The histogenesis of the pleomorphic adenoma is uncertain; so far several studies suggest that myoepithelial cells are responsible for the variable histomorphology of this type of neoplasm. At times, stroma is the predominant element. The purpose of the present review is to analyze the results reported in the scientific literature concerning immunomarkers expressed in the different stromal elements of pleomorphic adenoma.

Key words: Pleomorphic adenoma, Mixed tumor, Stromal variant

Introduction

Pleomorphic adenoma (PA) is a neoplasm of epithelial origin. The morpho-differentiation of the epithelial and myoepithelial cells in PAs are found in the parenchyma. However, the histology of this neoplasia encompasses a variety of cell morphologies and stromal areas, which may be chondroid, myxoid, fibrous, hyaline, adipose and osteoid (Barnes et al., 2005).

Transition is an important process in PAs. Transition refers to the differentiation of a cell from one type to another. In this neoplasm, transition occurs from an epithelial or myoepithelial cell to a stromal element and is called an epithelial-mesenchymal transition. This type of transition is due to the secretion of various proteins that favor PA histogenesis (Enescu et al., 2013).

PA accounts for 60% of salivary gland benign tumors. The average age of a patient is 46 years old, with a slight preference for the female gender. In 80% of cases, the tumor is located in the parotid gland; in 10% of cases in the submandibular gland and in the remaining 10% of cases in the minor salivary glands (Barnes et al., 2005). When a PA is located in minor salivary glands it can be found predominantly in the hard palate, the buccal mucosa and the upper lip (Jansisyanont et al., 2002).

In 1874, Minssen described PA as a mixed tumor based on the different patterns of the neoplasm. PA has been known over the years under several names, including: enclavoma, branchioma, endothelioma, enchondroma, benign mixed tumor and pleomorphic adenoma (Ledesma-Montes et al., 2002).

Over time, several researchers have presented varying views of the cellular origin of this neoplasia. Dardick et al. (1983) supported the theory that the myoepithelial cell (MeC) is responsible for the histogenesis of PA. They analyzed the role of reserve ductal cells and myoepithelial cells and concluded that a MeC that has displayed neoplastic alteration can potentially achieve multidirectional redifferentiation,

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which is relevant from the histogenic point of view to the origin of PA. These modified myoepithelial cells are located in the peripheral region of tubular glandular structures. They are considered a proliferative element that acquires different morphologies and secretory properties (Hübner et al., 1971; Dardick et al., 1982). However, in 1986 Batsakis reported that the purpose of MeC was still unknown and that it could play either a passive or active histogenic role.

Dardrick questioned the role of the ductal and myoepithelial reserve cells in PAs, while other researchers suggested they may originate in the mesenchymal cells (Quintarelli and Robinson, 1967; Mills and Cooper, 1981; Dardick et al., 1982, 1983; Enescu et al., 2013). This hypothesis was supported by the epithelium-mesenchyme differentiation process, which allows neoplastic epithelial cells to differentiate themselves from other cellular types of different lineage, resulting in the heterogeneity present in the PAs (Aigner et al., 1998; Langman et al., 2011; Enescu et al., 2013).

The present paper reviews existing the literature on immunohistochemically-expressed proteins in different stroma that have been found in PA and their role in the histogenesis of this type of neoplasia.

Myoepithelial and stromal component

Epithelial-myoepithelial carcinoma (EMC) and adenoid cystic carcinoma (ACC) share luminal (ductal) cell and peripheral ductal (myoepithelial) cell differentiation with PAs (Furuse et al., 2005; Nagao et al., 2012). The myoepithelial cells of salivary glands are located between the acinar luminal cells, interspersed ductal cells and basement membrane. They are MeC-differentiated into smooth muscle and epithelial cells. This is reflected in the ultrastructural appearance and the immunohistochemical phenotype (Raitz et al., 2003).

The morphologic diversity of PAs has been attributed to the differentiation of myoepithelial cells. The epithelium may form either ducts, cystic structures, or cells arranged focally or like sheets. Myoepithelial cells can exhibit a variable morphology and be observed as squamous keratinizing cells, basaloid cells, clear cells, and angular and spindle-shaped cells; sometimes they are round, displaying an eccentric nucleus and an eosinophilic hyalinized cytoplasm, mimicking plasmatic cells (plamacytoid cells) (Barnes et al., 2005).

There is controversy surrounding the histogenesis of the chondroid stromal, hyaline, myxoid, fibrous, osteoid and adipose areas typical of PAs (Nakanishi et al., 1990; Harrison and Auger, 1991; Nara et al., 1991; Neville et al., 2002). Margaritescu stated that PA stroma is formed from the proteins secreted by ductal neoplastic cells, as well as from the metaplastic transformation of myxoid zones and other stromal types (Mărgăritescu et al., 2005). For example, when stroma is fibrous, myoepithelial neoplastic cells are spindle-shaped and distributed between the collagen fibers. When stroma is hyaline, intensely eosinophilic hypo-cellular areas are observed. In myxoid areas, a pale matrix with hematoxylin affinity appears, while stellate and spindle-shaped cells can also be found. If chondroid elements are present, the stroma is similar to hyaline cartilage (Kusafuka et al., 2001a). Less frequently, osteoid (Nakano et al., 2007) and adipose tissues (Haskell et al., 2005) are found. The chondromyxoid stroma is a pathognomonic feature of this type of neoplasm (Mărgăritescu et al., 2005).

Transforming Growth Factor beta (TGF-β)

TGF-β is a superfamily that includes isoforms such as: TGF-β1, TGF-β2, and TGF-β3 of 25kDa (Lee, 1997; Kusafuka et al., 2001b). These are codified in 19q13, 1q41 and 14q24 chromosomes, respectively. They share a high homology in their amino acid sequence and are synthetized as inactive precursor proteins with 9 cysteine residues in the C-terminal (Barnard et al., 1990; Almazán, 2012).

TGF-β is a multifunctional cytokine involved in the development, differentiation, growth and apoptosis of most cells. Its signals are translated through transmembrane receptors with serine/threonine kinase activity that activates Smad2/Smad3 proteins (Lee et al., 2000; Almazán, 2012; Chen et al., 2012).

TGF-β induces *in vivo* chondroid tissue formation when implanted in the periostium (Iwasaki et al., 1993; Miura et al., 1994), and it participates in wound healing and endochondral ossification (Kusafuka et al., 2001b; Almazán, 2012).

Kusafuka analized the expression of TGF-β1, 2, 3 in 43 PAs; TGF-β1 was negative; TGF-β2 appeared in internal ductal cells in the nests of myoepithelial cells and in myxoid areas, but did not appear in chondroid areas; TGF-β3 was positive into neoplastic myoepithelial cells in squamous, myxoid and chondroid metaplastic areas and could be associated with ectopic chondrogenesis in PA (Kusafuka et al., 2001b).

Bone morphogenetic protein (BMP)

At least 20 types of BMP have been identified, each of them with a different function (Line et al., 1989). TBMP-2 to BMP-9 are members of the TGF-β superfamily. They derive from precursor polypeptide chains and may vary from 396 to 513 amino acids. Their precursors are formed by N terminal sequences and the C terminus segment. The main feature of the TGF-β superfamily is the preservation of amino acids in the C-terminal region with 7 complete cysteine residues, excepting BMP-8, which contains an eighth cysteine.

BMP-1 does not belong to the TGF-β family; it is located in chromosome 8 and does not induce bone formation. BMP-2 and 7 are located in chromosome 20; BMP-3 in chromosome 4 (4p14-q21); BMP-4 in chromosome 14 and BMP-5 and 6, in chromosome 6 (Lee, 1997; Kusafuka et al., 1998a, 1999a, 2001b).

BMP-6, 7 and 9 induce mesenchymatous and

osteogenetic differentiation originating in mesenchymal stem cells, with BMP-9 considered to have the greatest osteogenetic differentiation potential (Zhang et al., 2013). Neoplastic myoepithelial cells, located around chondroid areas, expressed BMP-2, suggesting that this protein plays an important role in the formation of these areas, while BMP-6 expression in lacunar cells could help in the preservation of the chondroid areas (Kusafuka et al.,1998a, 1999a). In addition, BMP-2 seems to regulate Runx2 expression during osteoblastic differentiation (Zhang et al., 2013).

Runx2

The Runx2 protein is also known as CBFA1 and is a key transcription factor associated with osteoblastic differentiation. It is encoded in the Runx2 gene in humans. Runx2 belongs to the Runx transcription factor family and has a Runt DNA-binding domain (Komori, 2008). It is the most important protein in osteogenesis and is located in chromosome 6p21. It is essential for osteoblastic differentiation and skeletal morphogenesis. The role of Runx2 in bone histogenesis has led to the idea that it may play an important role in the origin of osteoid areas in PA (Lee et al., 1992; Shigeishi et al., 2001; Nakano et al., 2007; Sivakumar and Niranjali, 2014).

Today it is widely accepted that the signaling pathways of TGF-β/BMP are involved in bone formation, activating the Smad protein complexes and translocating them to the nucleus in order to activate Runx2 and other transcription factors (Hamakawa et al., 1997; Arai et al., 2003). The production of autocrine BMPs is necessary to activate Runx2. Feedback is also required to stimulate osteoblastic genic expression (Zhang et al., 2013).

Keisuke Nakano et al. studied a case of PA bone formation to find the source of bone-forming cells. In their study they employed the S-100 protein, which was used to identify the modified myoepithelial cells and Runx2 and subsequently the cells responsible for the formation of bone tissue. The S-100 protein was expressed in the modified myoepithelial cells of stromal areas and Runx2 in osteoblasts and osteocytes (Nakano et al., 2007).

Osteopontin (OPN) and Osteonectin (OSN)

Osteopontin (OPN) is a multifunctional sialoprotein of 44-80 kDa, formed by 260-301 amino acids and encoded by the SPP1 gene located in the 4q (4q21-4q25) chromosome (Kusafuka et al., 1999b; Ramaiah and Rittling, 2008). It is found in the extracellular matrix and is abundant in bone, where it is synthetized by the pre-osteoblasts, osteoclasts and osteoblasts. In the osteoblasts, the mRNA expression of the OPN is regulated by different growth factors, including TGF-β and BMP (Ramaiah and Rittling, 2008). OPN is involved in the adherence and movement of osteoblasts and osteoclasts in bone cell junctions through integrins. It functions as a cellular binding protein and as a cytokine with a signaling function through two adhesion molecules: av β3-integrin and CD44 (Kusafuka et al., 1999b). OPN is also regulated by alkaline phosphatase activity (Aquino et al., 2013). Three variants of OPN - a, b, c-have been identified (Sivakumar and Niranjali, 2014).

OPN is expressed strongly in myxoid and hyaline stromas of PAs. Because OPN can bind to members of the integrin family in myxoid and hyaline stroma, it was able to induce production of basement membrane proteins via modified myoepithelial cells (Kusafuka et al., 1999b).

Osteonectin (OSN) is a glycoprotein of 43kDa, encoded by the SPARC gene (Bellahcene and Castronovo, 1995). SPARC (secreted protein rich in cysteine) also known as OSN or protein 40 (BM-40) is a member of the protein family in the extracellular matrix. Its function is to regulate cell-cell and cell-matrix interactions, although it is also involved in neoplastic transformation (Giachelli et al., 1997). OSN is involved in bone tissue mineralization and is present in active osteoblasts and young osteocytes. Therefore, it is a reliable marker of osteogenic cells and osteoblastoma and osteosarcoma (Bellahcene and Castronovo, 1995).

OSN in PA was expressed in the inner cells of tubular-glandular structures in the cytoplasm of the modified myoepithelial cells of myxoid areas and lacunar cells; there was no direct deposit in mesenchymal areas. OSN can induce proliferation in the inner ductal cells of tubular-glandular structures as well as induce the formation of chondroid and myxoid areas. OSN may be involved in cell proliferation and de novo synthesis of basement membrane (Kusafuka et al., 1999b).

OPN and OSN have been recently classified as malignity markers due to their expression in breast carcinoma and microcalcifications found in this neoplasm (Sunardhi-Widyaputra and Van Damme, 1994). Bovine OSN induces basement membrane molecule synthesis. In PA the myoepithelial modified cells release basement membrane molecules (Bellahcene and Castronovo, 1995), which are deposited to form the different stromal components (myxoid, hyaline, fibrous and chondroid).

Collagen family and its location

Neureiter et al. (1999) analyzed the tumoral matrix of 14 PAs of lesion free parotid and gland fragments as controls. They reported that collagen was negative in solid areas; ductal structures were surrounded by type IV collagen; stroma, fibrous areas and the capsule were positive for collagen I, III and VI; the chondromyxoid and chondroid areas were positive for type II collagen, which, together with Aggrecan, are expressed throughout the chondroid matrix around the lacunar cells; and lastly, Type VI Collagen surrounded the

chondrocytes (Table 1).

Chondromodulin I (ChM-I)

Chondromodulin (ChM-I) is a glycoprotein of 25 kDa (Klinger et al., 2011) formed by 121 amino acid residues and is encoded as the terminal C segment of a 335 amino acid precursor. The mature form of ChM-1 is secreted by chondrocytes after the proteolytic excision of a endoprotease precursor (Kusafuka et al., 2001a). ChM-1 promotes chondrocyte proliferation, proteoglycan synthesis and isogenous group formation (Klinger et al., 2011).

ChM-I expresses during hypertrophic chondrocyte proliferation in the growth plates of long bones (Line et al., 1989). It inhibits angiogenesis and, as a result, is responsible for the avascular nature of the cartilage (Kusafuka et al., 2001a). The expression and location of ChM-I has been compared to the expression of fibroblastic growth factor 2 (FGF-2) and CD34 to find relations in chondroid formation and hypo-vascularization in PA (Line et al., 1989). 100% of chondroid elements in PA expressed ChM-I in lacunar cells and neoplastic myoepithelial cells suggesting that this molecule may be related to hypovascularity and chondroid differentiation (Line et al., 1989; Kusafuka et al., 2001a).

Fibroblast Growth Factor (FGF) and its receptor

Fibroblast Growth Factor (FGF) is a polypeptide growth factor characterized by homologous sequences of amino acids with heparin binding. Its function is to promote angiogenesis and the mitogenic activity of epithelial, mesenchymal and neuronales cells (Kusafuka et al., 1998b). Some members of this growth factor carry out different functions during tissue development, scarring and tissue repair, in the maintenance of some cell types and in different pathological processes (Kato and Iwamoto, 1990; Kusafuka et al., 1998b). The basic fibroblast growth factor (bFGF) has also been discovered to be related to cartilage differentiation (Kusafuka et al., 1998b).

Fibroblastic Growth Factor 1 (FGF-1) has a strong binding affinity to bFGF, which contains heparan sulfate around the cellular surface, enabling bFGF and FGFR-1 (receptor fibroblast growth factor-1) binding, thereby regulating the chondrogenic activity of the growth plates in long bone. bFGFs also play an important role in cartilage differentiation (Kusafuka et al., 1998b).

FGFR-1 is a distinctive member of the tyrosine kinase receptor family; it has 3 immunoglobulin domains and a ligand binding domain (Allan et al., 1991). Iwamoto et al. reported that FGFR-1 is expressed in proliferative and mature chondrocytes within endochondral ossification in growth plates of large bones, but scarce expression is found in hypertrophic and calcified chondrocytes (Iwamoto et al., 1991).

In 1996 Myoken et al. found immunohistochemical overexpression of FGF-1, FGF-2 and FGFR-1 in myoepithelial cells of normal salivary glands (Myoken et al., 1996). Then, in 1998, Kusafuka studied aFGF, bFGF and FGFR-1 and found a high expression of bFGF and FGFR-1 in chondroid areas of PAs, showing their association to chondrocyte differentiation, because this growth factor promotes lacunar cell proliferation and proteoglycans production in the cartilage (Kusafuka et al., 1998b).

Discussion

Using RT-PCR, Kusafuka et al. analyzed BMPs-1, 2, 3, 4 and 7 and found excess mRNA expression of BMP-2 in PAs without mentioning the site. This mRNA expression was correlated to the immunohistochemical expression of type II collagen (Kusafuka et al., 1998a). It may be that BMP-2 could influence the histogenesis of chondroid stroma in PA, since this protein is involved in the chondrocyte redifferentiation process that prevents binding of nuclear factor kappa B (NF-ĸB) to the Col2a1 gene, thus promoting the production of type II collagen (Ghayor et al., 2000; Yu et al., 2009).

It is worth noting that the Col type IIA isoform found in undifferentiated mesenchymal cells and

Table 1. Distribution of collagen and other immunohistochemical markers in pleomorphic adenoma.

Pleomorphic adenoma	Normal parotid	Solid	Ductal	Fibrous	Myxoid	Chondroid	Hyaline
Collagen type I	+	(+)	+	++	+	(+)	
Collagen type II	-	-	-	-	-	++	
Collagen type III	+	(+)	+	++	(+)	(+)	
Collagen type IV	++ bm	+	++ bm	-	-	-	
Collagen type VI	+	(+)	+	++	+	++pc	
CD34				+++	+	-	+
ChM-1				-	++	+++	-
FGF-2				++	+++	+++	++
Aggrecan				+	+++	+++	+

Pc, pericellular; bm, basement membrane. Intensity of staining: -, negative; +, mild; ++, moderate; +++, intense; (), focally positive. Modified from Neureiter et al, 1999 and Kusafuka et al, 2001.

chondrogenic precursors is able to bind with members of the TGF-βs family (especially with TGF-β1 and BMP-2), creating signaling cascades through growth factors and thereby maintaining chondrocyte differentiation (Demoor et al., 2014). However, Kusafuka et al., concluded that TGF-βs do not participate in the formation of mesenchymal components, but rather that the BMP is related to chondrogenesis in this type of lesion (Kusafuka et al., 2001b). BMP-2 and TGF-β1 increase secretion of specific markers of cartilage and Col II, IX, XI, as well as preoteoglycans ((PGs) aggrecan) (Toh et al., 2005; Mehlhorn et al., 2007) (Fig. 1)

Collagen is encoded by the Col2a1 gene. Sox9, L-Sox5 and Sox6 act by regulating the chondrogenesis and the transcriptional activity of the gene, increasing the Col II expression primarily by Sox9. These three transcription factors also induce the Col2a1 gene expression Col IX and XI (Akiyama and Lefebvre, 2011; Lefebvre et al., 2001).

Another study analyzed BMP-6 in 23 cases of PAs (14 in major salivary glands and 9 in the palate); 10 of the 14 in the salivary glands showed chondroid areas and lacunar cells, which were both positive for BMP-6. The remaining 4 exhibited no chondroid areas. Of the 14 AP, BMP-6 showed scarce positivity in the internal epithelial cells of the tubular-glandular structures. No chondroid areas or BMP-6 expression appeared in the palate PAs (Kusafuka et al., 1999a).

TGF-β1 inhibits the terminal differentiation of chondrocytes, preventing the synthesis of Col X, osteocalcin, alkaline phosphatase, as well as osteoblast differentiation through the binding of Smad 2/3 to Runx2, and activating Sox9 (Ferguson et al., 2000; Wang et al., 2005; Hering et al., 2014). Reports reveal a lack of TGF-β1 expression in PAs (Kusafuka et al., 2001b), so another signaling pathway may be participating in the ossification of stromal elements in this neoplasia. However, there are few reported cases of osteoid formation. As concerns BMP-2, low doses induce high concentrations of chondrogenesis and osteogenesis. In locations of higher concentrations of BMP-2 there is a differentiation into hypertrophic chondrocytes with an expression of Col X through the

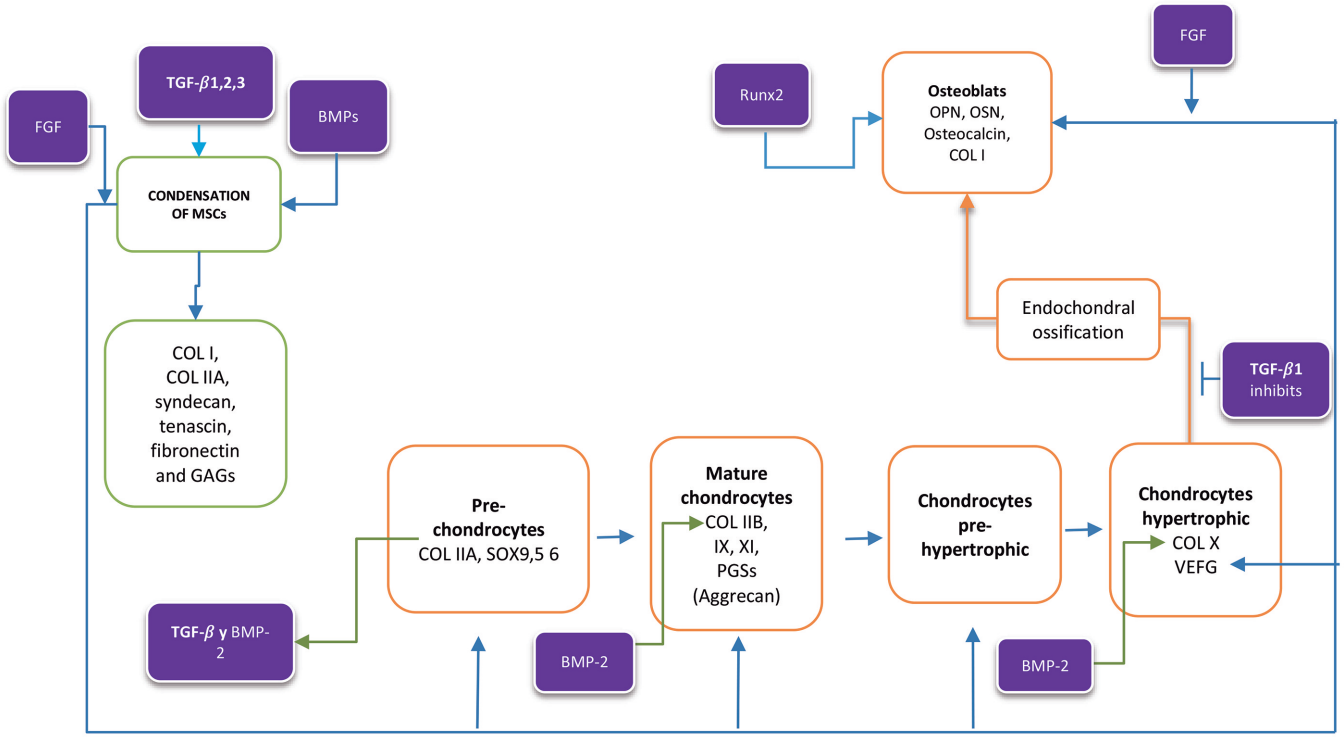

Fig. 1. Schematic overview of the role of growth factors at different stages of chondrogenesis. TGF-β1,2,3, BMPs and FGF induce condensation of MSCs. These MSCs secrete COL I, COL IIA (immature isoform COL II), adhesion molecules (syndecan, tenascin, fibronectin) and GAGs. These proteins induce differentiation into pre-chondrocytes, which express specific transcription factors for cartilage (SOX9,5 6) and COL IIA. COL IIA isoform enhances and activates TGF-β and BMP-2, respectively. BMP-2 induces synthesis of COL IIB (COL II mature isoform) and COL X in mature and hypertrophic chondrocytes. This last collagen facilitates Calcium deposits in the matrix where blood vessels are developed by VEGF, which in turn, promotes endochondral ossification. During differentiation into osteoblasts OPN, OSN, osteocalcin and COL I are secreted. TGF-β1 inhibits endochondral ossification. FGF stimulates proliferation, the differentiation of MSCs into chondrocytes, ossification and angiogenesis. Runx2 activates osteoblast differentiation. MCSs, mesenquimals stem cells; TGF-β, growth factor beta transformant; FGF, fibroblast growth factor; GAGs, glycosaminoglycans; BMP, bone morphogenetic protein; VEGF, vascular growth factor endothelium; OPN, Ostopontina; OSN, osteocalcin.

SMAD 1/5/8 pathway, activating Runx2 (Leboy et al., 2001; Valcourt et al., 2002).

In our review, few reported cases of PAs with osteoid were found (Chen et al., 1998; Shigeishi et al., 2001; Nakano et al., 2007). In 1952 Yates and Paget reported a case that considered endochondral ossification to be responsible for bone formation in this neoplasia (Shigeishi et al., 2001); however, Lee et al. related the osteoid matrix deposit to myoepithelial cell metaplasia (Lee et al.,1992). In 1997 Hamakawa et al. concluded that bone formation was caused by myoepithelial cells modified by endochondral ossification (Lee et al., 1992; Shigeishi et al., 2001). However, in a different view, Nakano et al. mentioned that Takeda and Yamamoto informed about a case in 1996 of a PA with bone formation and suggested that osteoblasts derive from the stromal metaplasia of dedifferentiated mesenchymal cells (Nakano et al., 2007). Shigeishi et al. emphasized that the formation of these areas are rare findings and concluded that stromal osteoid is caused by endochondral ossification within areas of chondroid tissue (Shigeishi et al., 2001). In 2003 Arai et al. attributed the origin of bone forming cells to the metaplasia of undifferentiated cells of true stromal tissue (Nakano et al., 2007). Therefore, in their conclusion Nakano et al. suggested that there are two theories about the origin of bone forming cells; one, that they originate from modified myoepithelial cells, and the other, that they originate from undifferentiated mesenchymal cells of true stromal tissue (Nakano et al., 2007). There is a possibility that tumor cells secrete BMP as metaplastic factors.

According to various studies, basement membrane-associated molecules and integrin-binding protein have been found in myoepithelial neoplastic cells and the hyaline stroma of PAs (Saku et al., 1988; Sunardhi-Widyaputra and Van Damme, 1994; Kusafuka et al., 1999b). The presence of integrins or integrin-binding proteins may indicate the ability to interact with different proteins, facilitating transition in this type of stromal tumor. Cheng et al. (1995) carried out an immuno-fluorescence study of two cell lines of the ACC from the salivary gland *in vitro* with IV collagen, laminin, heparan sulphate proteoglycan, entactin, and fibronectin. They found an expression of these proteins in the cytoplasm of parenchymal cells, concluding that the stroma of the ACC was the result of the secretion of these fibronectin basal membrane molecules. One might conclude based on this review that the hyaline stroma in PA is the product of the synthesis of basement membrane molecules, although studies must be carried out in the presence of other components in the basement membrane.

Through *in situ* hybridization and immunohisto-chemistry confirmation of collagen I, III and VI, Aigner et al. (1998) indicated a true fibroblast differentiation. Myxoid areas are often characterized by myoepithelial neoplastic cells with a stellate morphology and spindle-shaped cells that are immunohistochemically positive for GFAP, S-100 and vimentin. They are immersed in a stroma rich in mucopolysaccharides and scarce in collagen fibers (Mărgăritescu et al., 2005), which were positive for collagen I, III and VI but in lesser amounts than in the fibrous áreas (Aigner et al., 1998). The pathognomonic feature of PA is the presence of chondro-myxoid areas. Aigner et al. mentioned that myoepithelial neoplastic cells in myxoid stroma secrete mucopolysaccharides that encourage condroplastic differentiation, developing chondroid areas. These areas may be considered true main transition areas as they have an immunoexpression of ChM-1 and a lesser expression of CD34. Furthermore, the proteins secreted by mixoid stromal areas induce all other stromal elements found in this neoplasia (Fig. 2).

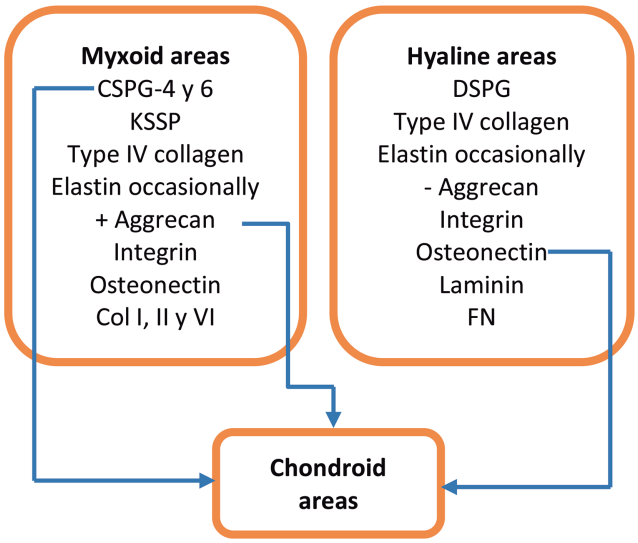

Fig. 2. Proteins present in AP myxoid and hyaline areas. The presence of FN in hyaline and mixoide areas could establish a migration pathway that would facilitate the expression of different binding proteins in this adhesion molecule. CSPG-4, 6 and KSSP were found in cartilaginous areas; this means that the expression of these proteins in the myxoid matrix could suggest that neoplastic stroma helps chondroid differentiation and that they can be considered essential transition areas for the cascade of protein signaling. This could be due to the fact that CSPG-4 and 6 are synthesized by immature mesenchymal cells that have differentiating ability. Proteins marked with arrows are necessary for the transition into chondroid stroma.

Table 2. Clones and dilution of primary antibodies.

Antibodies	Clone	Dilution
Vimentin	S-32322, Mouse monoclonal IgG1	1:100
Col2a1	Sc-28887, Rabitt polyclonal IgG	1:100
Col11a1	Sc-68853, Rabitt polyclonal IgG	1:100
Aggrecan	Sc-67513, Goat polyclonal IgG	1:100

*Santa Cruz® Biotechnology

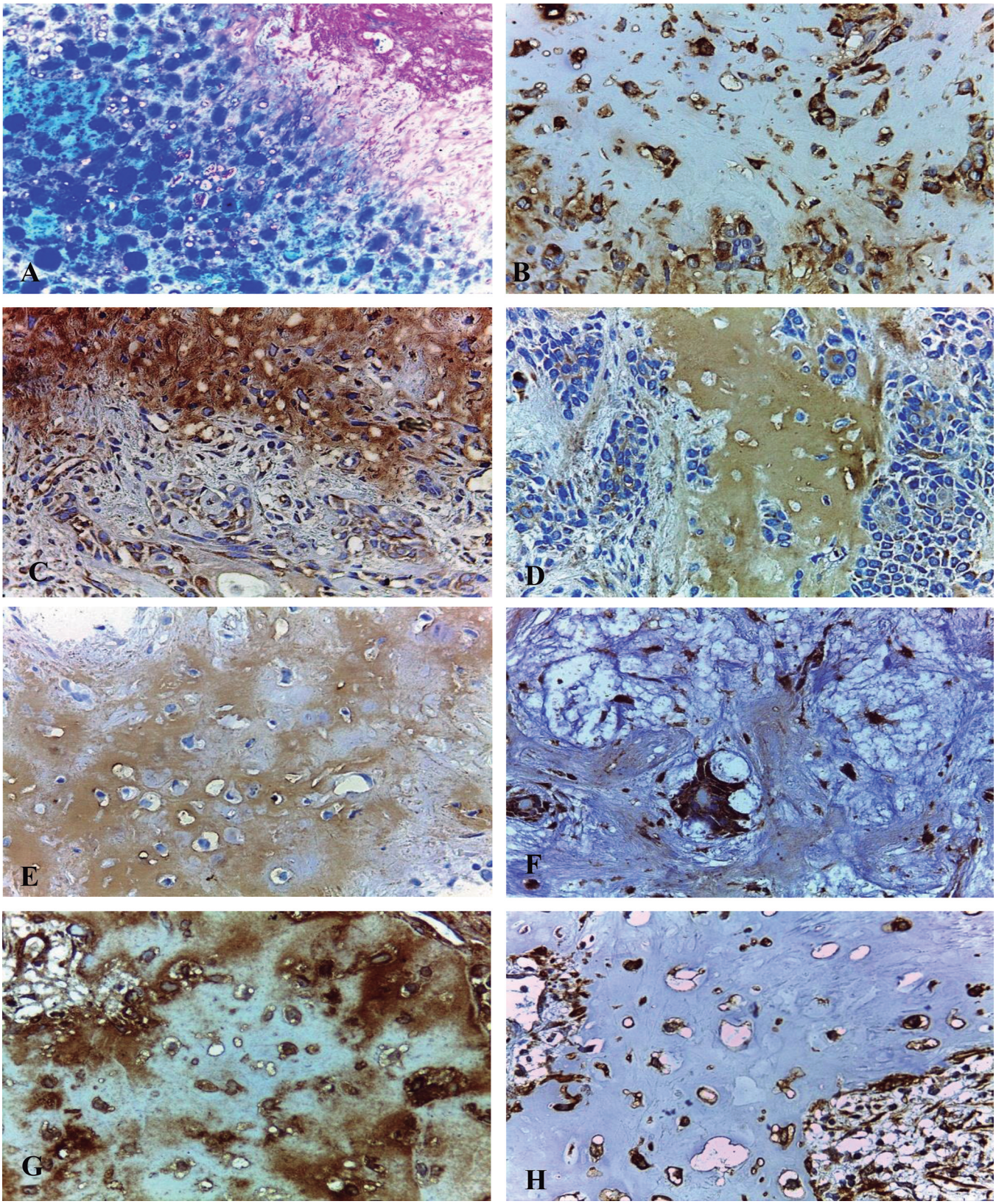

Fig. 3. Histochemistry and immunohistochemistry. **A.** Alcian Blue located in chondroid and myxoid matrix. **B, C.** Aggrecan. **B.** Expression of Aggrecan with high intensity in pericellular stroma and in cells with chondroid morphology. **C.** High intensity surrounding stromal cells with epithelioid and spindle-shaped morphology. **D, E.** COL II. Deposits on chondroid matrix, from high intensity to diffuse and moderate. **E.** COL II deposit chondroid matrix arranged diffusely localized around chondroid cells of moderate to high intensity. **F, G.** COL XI. High intensity cytoplasmic reaction in cells with stellate morphology and spindle-shaped on a myxoid stroma. **G.** Distribution of chondroid matrix and high and mild to moderate intensity immunoexpression chondroid cells. **H.** Vimentin. Reaction of high intensity in the cytoplasm of spindle-shaped cells located on the periphery of areas and lacunar chondroid cells. All antibodies were from Santa Cruz Biotechnology and all dilutions were at 1:100 (Table 2). A, x 100; B-H, x 400

As an example, we present some photomicrographs stained with antibodies available in our laboratory. A histochemical process using Alcian Blue discloses the presence of mucopolysaccharides in the chondroid and myxoid stroma. The immunohistochemical process reveals Aggrecan, Collagen II, XI and Vimentin (Fig. 3).

Conclusions

The present article purported to undertake a scientific literature review of protein expression in PA stromal varieties. According to the published data, we know that there is a loss of cytokeratins and SMA in cell differentiation into different stroma, as well as changes in the patterns of expression of the different collagen, such as Col IV. As a result, the intermediate filament acquires vimentin that is characteristic of mesenchymal tissue. The acquisition of different types of collagen such as Col II in myoepithelial and epithelial neoplastic cells, chondroid stroma and chondro-myxoid offer a basis for the stimulation of different growth and differentiation factors.

The TGF-β family possesses numerous ligands that are expressed in chondrocytes; many of the ligands are growth factors involved in chondrogenesis. The synthesis of TGF-βs can stimulate the expression of different transcription factors, initiating a cascade of signaling pathways, which may be responsible for the differentiation into the different morphologies present in this type of neoplasia. It would be interesting to observe the association pattern of Sox5, 6 and 9 with Col II in PA.

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