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A Subset of Cutaneous and Soft Tissue Mixed Tumors Are Genetically Linked to Their Salivary Gland Counterpart

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Abstract

Neoplasms morphologically similar to mixed tumors and myoepitheliomas of the salivary glands, under the broad concept of myoepithelial neoplasia, have recently been defined in the skin and soft tissue; however, to date, no data have supported a shared genetic background with their salivary gland counterpart. From a large body of research, it has been well established that rearrangement of *pleomorphic adenoma gene 1 (PLAG1)* leads to aberrant expression of its protein and is pathogenically relevant in the development of salivary mixed tumors. On the other hand, in soft tissue lesions, compelling evidence suggests that *EWSR1* is involved in a significant subset. To examine the hypothesis that there is a genetic link between these histologically similar tumors at different sites, we randomly selected 20 benign myoepitheliomas/mixed tumors of skin and soft tissue (10 cases each). Nineteen cases could be immunostained for PLAG1, of which 11 cases showed distinct nuclear staining with moderate or strong intensity in a significant number of cells. Interphase fluorescence in situ hybridization (FISH) for *PLAG1* was successfully performed in 11 cases (7 in skin, 4 in soft tissue) and was positive for gene rearrangement in 8 cases (5 in skin, 3 in soft tissue). All *PLAG1*-rearranged tumors, except one, had clear-cut ductal structures and were immunoreactive for PLAG1. In our series, tumors with *PLAG1* alteration shared a common morphologic phenotype characterized by prominent tubuloductal differentiation, suggesting that myoepithelial neoplasms with genuine salivary gland-like morphology, so-called soft tissue/cutaneous mixed tumors, are genetically related to their salivary gland counterpart.

Introduction

Mixed tumor (or pleomorphic adenoma) is the most common salivary gland neoplasm, comprising a mixture of epithelial and myoepithelial elements within a characteristic matrix-rich stroma produced by myoepithelial cells. Myoepithelial cells are specialized contractile cells in exocrine glands that regulate the outflow of secretions. These cells have a unique potential for divergent differentiation along both epithelial and mesenchymal lineages and are believed to play an integral role in the pathogenesis of mixed tumors (Hubner et al., 1971; Dardick et al., 1982). Along a continuous morphologic spectrum with mixed tumors, myoepitheliomas are tumors with predominantly myoepithelial differentiation without a discernible ductal component. Neoplasms morphologically analogous to mixed tumors or myoepitheliomas, under the broad concept of “myoepithelial neoplasia”, may also occur at extra-salivary locations, including the lung, viscera, bone, and notably the skin and soft tissues. Outside the salivary gland, however, myoepithelial neoplasia is a newly introduced concept in the literature (Kilpatrick et al., 1997) and only recently have the clinicopathologic

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characteristics of such lesions in the skin and soft tissues been established (Kilpatrick et al., 1997; Hornick and Fletcher, 2003, 2004).

Sweat glands, similar to salivary glands, are exocrine tubuloacinar units with both secretory and excretory function and serve the role of thermoregulation. The apparent morphologic similarities between these two types of exocrine glands, and at least a subset of their neoplastic counterparts, raise the distinct possibility of a shared genetic background between their respective neoplasms. Some evidence in support of this notion has been provided by common fusion transcripts in a subset of mucoepidermoid carcinoma of the salivary gland and cutaneous hidradenoma, specifically *CRTC1-MAML2* and *EWSR1-POU5F1* (Behboudi et al., 2005; Moller et al., 2008). Recently, the *MYB-NFIB* chimeric gene was discovered in adenoid cystic carcinoma of the salivary gland and dermal cylindroma, further supporting a possible genetic association between them (Fehr et al., 2011). Tumors with the closest morphologic equivalence in the skin and salivary glands are mixed tumors (also known as chondroid syringoma in skin), but to date, no data in support of a common genetic profile have been provided.

From a large body of research, it has been well established that the target gene in the development of pleomorphic adenoma is the *pleomorphic adenoma gene 1 (PLAG1)*. *PLAG1* is a proto-oncogene located in the chromosome band 8q12 (Kas et al., 1997; Voz et al., 2000b). Elevated activity of *PLAG1* primarily results from recurrent translocations that lead to upregulation of the *PLAG1* gene (Kas et al., 1997, 1998; Voz et al., 1998, 2000b; Astrom et al., 1999). Contrary to the extensive cytogenetic and molecular genetic studies of pleomorphic adenomas over the course of the past 2.5 decades (Mark and Dahlenfors, 1986; Bullerdiek et al., 1993; Kas et al., 1997, 1998; Voz et al., 1998, 2000a, 2000b, 2004; Astrom et al., 1999; Asp et al., 2006; Van et al., 2007), few studies have focused on exploring the genetics of myoepithelial tumors in soft tissues (Hallor et al., 2008; Antonescu et al., 2010). This is partly because of the novelty of the myoepithelioma concept in soft tissue (where there is no evident normal cellular counterpart), and partly due to the rarity of these tumors at extra-salivary sites and the difficulty in classifying them. Hallor et al. analyzed whole genome DNA copy number imbalances and the genomic status of the *PLAG1* gene in 5 soft tissue myoepitheliomas (Hallor et al., 2008). They found a heterogeneous genetic profile and only one significant recurrent aberration in these tumors, the *CDKN2A* tumor suppressor gene deletion (Hallor et al., 2008). They detected *PLAG1* rearrangement in a single soft tissue tumor; however, given the previous history of salivary pleomorphic adenoma in the patient, it was not clear whether the soft tissue tumor was a primary or a morphologically benign metastasis from the patient's prior salivary gland lesion. In conclusion, the study could not provide convincing evidence in support of a genetic commonality between these tumors (Hallor et al., 2008).

Here we present a series of benign mixed tumors and myoepitheliomas of the skin and soft tissues and demonstrate abnormality of the *PLAG1* gene in a significant proportion of them. In our samples, the cytogenetically rearranged tumors shared a common morphologic phenotype, characterized by clear-cut tubuloductal differentiation, suggesting that the subset with true salivary gland-like morphology, so-called mixed tumors of skin and soft tissue, are genetically linked to their salivary gland counterpart.

Materials and Methods

Twenty cases of benign myoepithelioma or mixed tumor of the skin and soft tissue were retrieved from the consultation files of one of the authors (CDMF). Ten tumors were centered in the dermis/ superficial subcutis and 10 arose in the deep soft tissue. Eleven cases had been included in a prior study (2 in skin, 9 in soft tissue) and been analyzed for *EWSR1*

rearrangement previously (Antonescu et al., 2010). The *PLAG1* gene status and *PLAG1* expression for each case were evaluated blinded to the results of prior *EWSR1* gene analysis by two of us (AB and JDD). All cases met the current diagnostic criteria (Hornick and Fletcher, 2003), with a supportive immunoprofile, requiring expression of S-100 protein and/or GFAP along with cytokeratin, EMA, or both. Labeling for myogenic markers could be variable. The tumors occurred in 11 females and 9 males, ranging from 6 months to 84 years old (median, 55.5 years old) and involved the following anatomic regions: lower extremities (10 cases), upper extremities (3 cases), trunk (3 cases), and head and neck (4 cases) (Table 1). The tumors in the head and neck areas were located in the scalp and forehead, with no possible connection to salivary glands.

Immunohistochemistry for *PLAG1*

Formalin-fixed, paraffin-embedded tissue from 20 cases were cut into 4 μ m-thick sections and immunostained with a monoclonal *PLAG1* antibody (clone 3B7, 1:50; Novus, Littleton, CO), using heat-induced epitope retrieval in citrate buffer for 30 minutes and the Bond Polymer Refine Detection System (Leica Microsystems, Bannockburn, IL) after 2 hours of antibody incubation at room temperature. Positive controls were included in each run, using sections of a classic pleomorphic adenoma and a lipoblastoma. Immunostains were scored semiquantitatively as follows: “0”, no positive cells; “rare”, rare to <5% positive cells; “1+”, 5-25% positive cells; “2+”, 26-50% positive cells; and “3+”, >50% positive cells. The intensity of nuclear staining was graded as “faint”, “moderate”, and “strong”.

Fluorescence In situ Hybridization Study for *PLAG1*

Interphase fluorescence in situ hybridization (FISH) for *PLAG1* was developed by a dual-color breakapart probe set using bacterial artificial chromosome (BAC) clones chosen according to the UCSC Genome Bioinformatics database (<http://genome.ucsc.edu>) to include the RP11-22E14 clone, flanking the 3' end, and the RP11-1130K23 clone, flanking the 5' end of the *PLAG1* gene (BACPAC Resources, Oakland, CA). DNA was isolated using a modified Qiagen (Valencia, CA) plasmid extraction protocol. The RP11-22E14 sequence was labeled with AlexaFluor 488 (green fluorochrome) and the RP11-1130K23 sequence with rhodamine (red fluorochrome). The labeled products were co-precipitated with Cot-1 DNA and salmon testes DNA and then resuspended in Tris buffer. Dual-color FISH was performed on 4 μ m-thick formalin-fixed, paraffin-embedded tissue sections. All probe mixtures were diluted in hybridization buffer and co-denatured with the target DNA on a slide moat at 90°C for 12 minutes. The slides were incubated overnight at 37°C on a slide moat and then washed in 4 M urea and 2 $\times$ SSc at 25°C for 2 minutes. After the post-hybridization wash, nuclei were counterstained with DAPI (Vector Labs, Burlingame, CA). The FISH sections were examined using an Olympus (Center Valley, PA) BX51 fluorescence microscope equipped with a 100-watt mercury lamp and FITC, rhodamine, and DAPI filters. Images were captured and processed with an exposure time ranging from 0.1 to 2 seconds for each fluorochrome using CytoVision v4.5 software (Leica Biosystems, Richmond, IL). A normal control produced two pairs of overlapping green and red (yellow) signals in each nucleus. For each sample, 200 nuclei were examined. Material for FISH studies was available for 13 cases, including 8 tumors in the skin and 5 tumors in the soft tissue.

Results

Histologic Features

Morphologically, the tumors were generally well circumscribed and lobulated or, at most, had minimally infiltrative margins. Most tumors were composed of an admixture of epithelioid, ovoid, or spindle cells arranged in strands, ducts, islands, or solid sheets within

a chondromyxoid or hyalinized stroma (Figures 1 A1-A4 and 2 A1-A3). Many tumors had a mixture of architectural patterns, frequently in trabecular and reticular arrangements. Foci of chondroid metaplasia were commonly present (Figure 1 B4). Distinct ductular or tubular structures with sharply defined luminal borders were evident in 11 of 20 cases. No tumor had the morphological appearance of parachordoma. None of the tumors showed overt features of malignancy such as marked nuclear pleomorphism, prominent nucleoli, more than occasional mitosis, or necrosis. A few tumors contained scattered larger cells with degenerative-type nuclear atypia without elevated mitotic activity.

PLAG1 Immunohistochemistry

The immunohistochemical findings are summarized in Table 1 and representative images are shown in Figures 1 B1-B4 and 2 B1-B3. In summary, 6 cases did not stain for PLAG1, and 1 case failed to stain due to technical difficulty. Thirteen cases expressed PLAG1; in 1 case the staining was faint, and in 12 cases it was unequivocal with moderate or strong intensity and the following scores: “rare” in 1 case, “1+” in 3 cases, “2+” in 1 case, and “3+” in 7 cases. Overall, 11 of 19 cases (58%) showed distinct nuclear staining in a significant proportion of cells (1+ or more; moderate or strong). All tumors with distinct tubular differentiation were positive for PLAG1 (1+ or more). In tumors without ducts, PLAG1 was negative or positive in only rare cells, except in one case that was only weakly positive.

PLAG1 expression was seen in all types of tumor cells. The staining was generally diffuse in myoepithelial cells but more sporadic and sometimes absent in the inner epithelial cells of tubuloductal structures (Figure 1 B2), similar to the discriminative pattern of staining described in pleomorphic adenoma (Debiec-Rychter et al., 2001).

PLAG1 FISH

The FISH findings for each individual case are summarized in Table 1 and representative images are demonstrated in Figures 1 C1-C4 and 2 C1-C3. Briefly, the interphase FISH was positive for gene rearrangement in 8 cases (5 in skin; 3 in soft tissue), negative in 3 cases (2 in skin; 1 in soft tissue), and indeterminate due to poor hybridization in 2 cases (1 each in skin and soft tissue). In cases considered to be positive for gene alteration, split signals were detected in 46%-96% of nuclei (median, 70%). Of 8 *PLAG1*-rearranged tumors, 4 cases had one pair of split signals and one intact gene; in two of these (1 each in skin and soft tissue), the pattern was consistent with a balanced translocation and in other two (both in soft tissue), it was suggestive of an inversion/ intrachromosomal rearrangements. The FISH pattern in 1 case in skin suggested an interstitial deletion, and in 3 cases (all in skin), the pattern was consistent with an unbalanced translocation, showing one intact gene and one isolated green signal. Overall, 8 of the 11 cases that could be successfully analyzed by FISH (73%) were positive for *PLAG1* gene rearrangement. All FISH-positive tumors, except one, had distinct ductal structures and were immunoreactive for PLAG1. Gene rearrangement by FISH could be observed in inner epithelial cells of ducts, including those that were sporadically PLAG1 positive.

Discussion

Our study revealed aberration of *PLAG1* in a subset of mixed tumors of skin and soft tissue, in common with salivary gland mixed tumors, suggesting a shared pathogenetic mechanism. From earlier research and the karyotypic analysis of several hundred pleomorphic adenomas (Mark and Dahlenfors, 1986; Mark et al., 1988; Sandros et al., 1990; Bullerdiek et al., 1993), it has been shown that approximately 70% of salivary pleomorphic adenomas have structural chromosomal abnormalities and 30% have an apparently normal karyotype (Stenman, 2005). The cytogenetic anomalies include recurrent translocations involving the

PLAG1 gene in 8q12 (in 39%), translocations affecting *HMGA2* in 12q14~15 (in 8%), and sporadic clonal alterations (in 23%) (Kas et al., 1997; Geurts et al., 1997; Stenman, 2005). The pathogenic involvement of *PLAG1* in pleomorphic adenoma, however, is broader than what can be inferred from the above findings. Northern blot analysis has shown that *PLAG1* is overexpressed in nearly all pleomorphic adenomas, including those with a normal karyotype and those with *HMGA2* rearrangements (Astrom et al., 1999; Stenman, 2005). Alternative pathways, such as gene mutation, increased gene copy number, polysomy of chromosome 8, and cryptic gene modifications have been suggested as potential mechanisms responsible for gene overexpression in tumors without apparent 8q12 aberrations (Astrom et al., 1999; Stenman, 2005).

PLAG1 is a developmentally regulated gene, the activity of which is mainly restricted to fetal life (Hensen et al., 2004). The gene protein product, PLAG1, is a nuclear transcription factor that binds to DNA at specific sites (Voz et al., 2000a). Microarray screening for *PLAG1* target genes found 47 genes induced by *PLAG1*, among which a large class of growth factors, including the human *insulin-like growth factor 2 (IGF2)* (Voz et al., 2004). The *PLAG1* oncogenesis is thought to be mediated, at least in part, by upregulation of *IGF2* and its mitogenic signaling pathway (Voz et al., 2000a, 2004).

Translocations involving *PLAG1* lead to gene overexpression, via promoter swapping between *PLAG1* and one of its partners (Kas et al., 1997; Voz et al., 2000b). A common feature among several potential *PLAG1* partner genes is ubiquitous expression in all fetal and adult tissues (Stenman, 2005). The translocation breakpoint typically occurs in the 5' non-coding regions of both genes, leaving the coding sequences intact (Kas et al., 1997; Voz et al., 2000b). As a result, *PLAG1* is brought under the control of an active promoter, leading to overexpression of *PLAG1* and downregulation of its partner gene (Kas et al., 1997; Voz et al., 1998). The preferential candidate gene in nearly 50% is *CTNNB1* (*catenin (cadherin-associated protein), beta 1*), the gene coding for beta-catenin in 3p21 (Kas et al., 1997). The second common gene is *LIFR* (*leukemia inhibitory factor receptor*), mapped to 5p13 (Voz et al., 1998). Two other partner genes have been found in the same chromosomal band, namely *TCEA1* (*transcription elongation factor A (SII)*), situated 2 Mb centromeric to *PLAG1*, and *CHCHD7* (coiled-coil-helix-coiled-coil-helix domain containing 7), located only 500 bp telomeric to *PLAG1* (Astrom et al., 1999; Asp et al., 2006). Rearrangements in partnership with the latter two do not lead to gross chromosomal anomalies and are undetected by conventional karyotyping and likely also by standard FISH. The possibility of gene involvement, therefore, is not completely excluded by FISH studies at routine resolutions. A pattern suggesting a possible inversion/ intrachromosomal gene alteration, however, could be detected in 2 of our cases, in which the split signals were observed at a constantly short distance apart.

A common genetic aberration or even a shared fusion transcript does not necessarily indicate a pathogenetic relatedness between two lesions in the absence of proper morphologic association. As known in several types of solid tumor, a gene defect may be shared by tumors of entirely unrelated histogenesis. The oncogenic role of *PLAG1*, for instance, is not exclusive to pleomorphic adenoma and has been implicated in the development of lipoblastoma. The oncogenic mechanism in lipoblastoma is similar, but the translocation is in collaboration with two candidate genes that have never been found in pleomorphic adenoma, namely *COL1A2* (*collagen, type I, alpha 2*) and *HAS2* (*hyaluronan synthase 2*) (Astrom et al., 2000; Hibbard et al., 2000). Given the shared morphologic similarities and line of differentiation in salivary and skin/ soft tissue mixed tumors, the discovery of *PLAG1* rearrangement in both, on the other hand, provides good evidence of a shared pathogenetic linkage between them. Although in our study of cutaneous and soft tissue mixed tumors, the complete characteristics of the fusion genes was not further investigated, we speculate that

the pathogenic pathways are comparable to those in salivary gland mixed tumors. The immunohistochemical expression of *PLAG1* found in nearly all *PLAG1*-rearranged tumors in our series, for example, indicates that, as in pleomorphic adenoma, translocations involving *PLAG1* lead to gene activation.

On the other hand, there are some histologically undeniable differences between mixed tumors at these sites. For instance, the prognostically relevant histologic criteria for malignancy differ to some extent between them; i.e., in tumors primary to the salivary gland, infiltrative growth is the foremost criterion for malignancy; whereas in soft tissue myoepitheliomas, cytologic atypia and nuclear features appear to have the most predictive value in defining malignancy (Hornick and Fletcher, 2003). It is, therefore, a fair statement that definite proof for a unifying pathogenesis is more convincingly supported by finding similar partner genes, although there are possibly several involved.

The histogenetic origin of mixed tumor of the salivary gland is thought to be the intercalated duct and its conceptual undifferentiated reserve cells, akin to embryonic development (Batsakis, 1980). An alternative hypothesis, however, could argue a myoepithelial cell with multidirectional differentiation capacity and clonal proliferation as a possible precursor. Although the normal structural origin of myoepithelial neoplasms in extra-salivary sites, including deeply seated soft tissues, is not always apparent (and perhaps not required), those arising in the skin or more superficial soft tissues are generally thought to be associated to myoepithelial cell constituents in eccrine and apocrine glands. The coiled secretory segment of sweat glands, often located in the dermis or at its interface with the subcutis, is structurally similar to the intercalated duct segment of salivary glands, having a discontinuous layer of myoepithelial cells in its structure. Bearing in mind that this segment is sometimes found deep in the subcutaneous fat, it is not implausible that, in at least some soft tissue mixed tumors, the sweat gland may be the potential structure of origin.

The plasticity of myoepithelial cells and the variable proportions of stromal and cellular components account for the phenotypic diversity of myoepithelial tumors of soft tissue, similar to their salivary gland counterpart. In light of the cytoarchitectural heterogeneity, these neoplasms have been morphologically categorized under one umbrella with various terminologies. At one end of the spectrum, “mixed tumor” is a term more properly assigned to neoplasms with obvious ductular differentiation, encompassing chondroid syringoma. The latter is often reserved for invariably benign and smaller, more superficially located dermal mixed tumors. At the opposite end of the spectrum is “myoepithelioma,” a tumor generally lacking ductal differentiation. Parachordoma is widely regarded as a morphologic subtype composed predominantly of large epithelioid cells with clear to pink vacuolated cytoplasm. The exact assignment of tumors to these categories based on histologic parameters, however, can sometimes be difficult if not impossible due to the extent of morphologic overlap. In addition, in contrast to the phenotypic diversity, no discriminatory immunoprofile or genetic characteristics to justify recognition of each as a distinct pathologic entity have been found as yet. For these reasons, these tumors have been regarded as the morphologic continuum of a single class of tumors having in common evidence of myoepithelial differentiation.

In our series, most *PLAG1*-rearranged tumors had a shared morphologic feature, characterized by distinct tubuloductal structures, consistent with the category of mixed tumors. This finding raises an intriguing question, as to whether the true salivary gland-like cutaneous or soft tissue mixed tumors are genetically distinct from those at the other end of the morphologic spectrum, namely myoepitheliomas. We believe that the evidence to support such notion is still unsettled. In our study, only 3 cases that could be successfully analyzed by FISH were negative for *PLAG1* gene alteration. Although all 3 showed

morphologic features more in keeping with myoepithelioma than mixed tumor, the sample size is too small to conclude a perfect morphologic and genetic correlation. In addition, exception was found in a single case in the skin in which *PLAG1*-rearrangement was confirmed cytogenetically without histologic evidence of ductal differentiation in the sample. Although one could argue that sampling error might have caused this morphologic interpretation, we cannot be certain in this regard.

A prior study by Antonescu et al. provided evidence for the role of *EWSR1*, in partnership with *PBX1*, *ZNF444*, or *POU5F1*, in a substantial subset of benign and malignant soft tissue and visceral myoepithelial tumors (Antonescu et al., 2010). *EWSR1* alteration was initially reported in isolated case reports (Gleason and Fletcher, 2007; Brandal et al., 2008, 2009), but was subsequently investigated thoroughly by this group, in a study of 66 extra-salivary, benign and malignant myoepithelial neoplasms at various sites, showing *EWSR1* rearrangement by FISH in 45% of them (Antonescu et al., 2010). It is notable that in the above study, all cases with ductal structures were negative for *EWSR1* alteration, a conclusion that complements our findings with *PLAG1* and support the notion of different genetics for the two morphologic categories of myoepithelial neoplasia. We were able to refer back to the FISH results for *EWSR1* from the prior study and did not identify any case with concurrent *EWSR1* and *PLAG1* changes. We, therefore, speculate that abnormalities of the two genes are likely to be mutually exclusive. Of note, one myoepithelioma with genetically confirmed *EWSR1* disruption in our study was found to be weakly positive for *PLAG1*. We did not have sufficient material to evaluate the tumor for *PLAG1* rearrangement or to enumerate chromosome 8; nonetheless, we do not believe it likely that gene rearrangement was responsible for equivocally expressed *PLAG1* in this case and hypothesize that, if it was truly immunoreactive, other postulated mechanisms for aberrant *PLAG1* expression, such as polysomy of chromosome 8, could have been responsible. While *PLAG1* rearrangement has not been described in any tumor other than lipoblastoma and pleomorphic adenoma, *PLAG1* overexpression without gene alteration has been demonstrated in hepatoblastoma and a subtype of acute myeloid leukemia (Zatkova et al., 2004; Landrette et al., 2005). Genomic copy gain or amplification are thought to be the potential mechanisms in some hepatoblastomas (Zatkova et al., 2004).

In conclusion, the results of our experiment in conjunction with those in studies on *EWSR1* suggest that there are at least two genetic categories of mixed tumor or myoepithelioma at locations outside the salivary glands: those with *EWSR1* and those with *PLAG1* rearrangement. In the study of Antonescu et al., over half of the cases with *EWSR1* alteration were found to be histologically malignant, whereas all *EWSR1*-negative cases were benign tumors (Antonescu et al., 2010). However it would be premature and unjustified (as yet) to conclude that the genetic category with *PLAG1* alteration defines tumors of invariably benign behavior. Although the concept of carcinoma ex-pleomorphic adenoma has only occasionally been described in soft tissue mixed tumor (Hornick and Fletcher, 2004), the potential for malignant transformation analogous to what is observed in the salivary glands may also exist at other sites. Future studies focusing on the *PLAG1* status of malignant myoepithelioma may provide further insights.

In summary, we have shown that some tumors with myoepithelial differentiation in the skin or soft tissue have a common genetic alteration with their salivary gland counterparts and may originate from clonal proliferation of a similar type of precursor cell. We hypothesize that aberrant *PLAG1* expression, as a result of gene rearrangement, can trigger histologically similar tumors, namely mixed tumors, at different sites. The hypothetical cell of origin with *PLAG1* alteration could be a myoepithelial cell with the capacity for multidirectional differentiation or an undifferentiated stem cell in the exocrine ducts. Broadening the molecular analysis and characterizing the fusion transcripts may provide clearer insight into

the relationship between salivary gland and extra-salivary mixed tumors. Also, the current data suggest that, among the class of tumors with myoepithelial differentiation in the skin and soft tissue, those with evidence of *PLAG1* rearrangement appear to be morphologically closer to their homologous tumors in the salivary gland than the lesions described previously with *EWSR1* rearrangement.

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Figure 1.

Morphologic features (H&E), PLAG1 immunohistochemistry, and interphase FISH for *PLAG1* in 4 cases of benign mixed tumors of skin. 1 (case 1), 2 (case 6), 3 (case 9), and 4 (case 10). A1-A4: Epithelioid, spindled and plasmacytoid cells are arranged in various architectural patterns in association with a hyalinized, myxoid or cartilaginous stroma. Distinct tubular structures are discernable in all four cases. B1-B4: Most tumor cells show strong nuclear staining for PLAG1. In case 6, the PLAG1 staining pattern is variable. Cells surrounding the tubuloductal structures are diffusely positive, whereas luminal epithelial cells are more sporadically stained. C1: One intact yellow (overlapping green and red) signal and one red signal. Loss of the green signal, flanking the 3' tail of *PLAG1*, suggests gene rearrangement with an interstitial deletion. C2: One intact yellow signal and one single green signal; the pattern indicates an unbalanced translocation with loss of red signal, flanking the *PLAG1* promoter region. C3: One intact gene and one pair of split red and green signals with an additional one or two copies of green signal; the pattern suggests an unbalanced translocation with copy gain. C4: One pair of split green and red signals, suggestive of a balanced translocation.

Figure 2.

Morphologic features (H&E), PLAG1 immunohistochemistry, and interphase FISH for *PLAG1* in 3 soft tissue myoepitheliomas. 1 (case 17), 2 (case 19), 3 (case 20). A1-A3: Mixed tumors of soft tissue showing a hyalinized or chondromyxoid matrix and well defined ductal structures. B1-B3: All 3 cases show strong nuclear staining for PLAG1 in the majority of tumor cells. Note the absence of staining in endothelial cells, supporting stromal cells, and lymphocytes. C1 and C3: In both cases, the split signals were observed at a constantly short distance apart, suggesting an inversion or intrachromosomal rearrangement. C2: FISH showing most cells with one intact yellow signal, and one pair of split red and green signals, suggestive of a balanced translocation.

Demographics, histologic, and cytogenetic findings in 20 benign skin and soft tissue mixed tumors/ myoeipitheliomas.

Table 1

Case No.	Age (Yo)	Sex	Location	Tubular Diff	PLAG1 IHC (score/intensity)	PLAG1 FISH	EWSR1 Status *
1	55	F	Skin, foot	Y	3+/strong	Pos (with interstitial Del)	UK
2	70	F	Skin, back	Y	1+/intermediate	Ind	UK
3	39	M	Skin, chest wall	N	Ind	Pos (Unbal)	UK
4	60	M	Skin, upper arm	N	0	NP	UK
5	36	F	Skin, arm	N	0	Neg	UK
6	84	M	Skin, forehead	Y	2+/strong	Pos (Unbal)	Neg
7	0.5	M	Skin, scalp	N	0	Neg	UK
8	66	F	Skin, lower leg	Y	3+/strong	NP	Neg
9	64	F	Skin, scalp	Y	3+/intermediate	Pos (Unbal) & copy gain	UK
10	56	F	Skin, scalp	Y	3+/strong	Pos	UK
11	32	M	ST, ankle	N	2+/faint	NP	Pos
12	73	F	ST, thigh	Y	1+/intermediate	NP	Neg
13	41	M	ST, foot	N	Rare/intermediate	Ind	Neg
14	53	F	ST, abdominal wall	N	0	NP	UK
15	34	M	ST, thigh	N	0	NP	Neg
16	58	F	ST, groin	Y	1+/intermediate	NP	Neg
17	62	M	ST, lower leg	Y	3+/strong	Pos (?Inv)	Neg
18	20	M	ST, foot	N	0	Neg	Pos
19	67	F	ST, axilla	Y	3+/strong	Pos	Neg
20	52	F	ST, foot	Y	3+/strong	Pos (?Inv)	Neg

Abbreviations: Del, deletion; Diff, differentiation; F, female; Ind, indeterminate; Inv, inversion; M, male; N, No; Neg, negative; NP, not performed; Pos, positive; ST, soft tissue; UK, status unknown; Unbal, unbalanced translocation; Y, yes; Yo, years old.

* Information obtained from Antonescu et al., 2010.