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Morphological features and genetic background in ectomesenchymal chondromyxoid tumor: A systematic review

Raquel Helena Junia de Souza[1], Fernanda Aragão Felix[1], Flávia Martins Vasconcelos Filiú[1], Witalo Pereira de Jesus[1], Felipe Paiva Fonseca[1], Ricardo Santiago Gomez[1,2], Lucas Guimarães Abreu[3] and Sílvia Ferreira de Sousa[1]

[1]Department of Oral Surgery, Pathology and Clinical Dentistry, School of Dentistry, Federal University of Minas Gerais, [2]Medical School, Faculty of Medical Sciences of Minas Gerais and [3]Department of Pediatric Dentistry, School of Dentistry, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

Summary. Background. Ectomesenchymal chondromyxoid tumor (EMCMT) is a rare neoplasm that mainly affects the tongue and harbors recurrent, although not exclusive, gene fusions. Owing to its rarity, overlapping features with other tumors may lead to challenges in the microscopic diagnosis. We aimed to perform a systematic review focusing on the histomolecular findings of EMCMT of the oral and maxillofacial region and to evaluate the possible association between microscopic features with the genetic background.

Methods. An electronic search was made on PubMed, Web of Science, Scopus, Ovid, and Embase. Clinicopathological, immunohistochemical, and molecular data were retrieved.

Results. Overall, 114 cases from 53 articles on EMCMT were analyzed. Histologically, EMCMT was described as demarcated (84.2%), lobulated (66.7%), reticulated (51.8%), and arranged in sheets, cords, and strands (42.9%), with 73.7% of lesions with spindle-shaped cells. Myxoid stroma (88.6%), chondroid areas (60.5%), chondromyxoid stroma (57.0%), and fibrous septae (42.9%) were also tumor-outlined features. The most expressed markers were vimentin (100.0%), cyclin D1 (100.0%), GFAP (88.5%), NSE (87.5%), S100 (86.5%), CD56 (76.9%), and CD57 (76.5%). The *RREB1-MRTFB* fusion was detected in 91.0% of the cases investigated and *EWSR1* rearrangements in 17.4%. The presence of the fusion *RREB1::MRTFB* or chromosome alterations in the *EWSR1* gene were not highly specific to the morphological features of EMCMT.

Conclusion. This study provides a comprehensive summary of the clinicopathological, immunohistochemical, and molecular characteristics of EMCMT, aiding in a more accurate microscopic diagnosis of this rare tumor.

Key words: Ectomesenchymal chondromyxoid tumor, Immunohistochemistry, Oral neoplasm, Fusion gene, Rearrangement

Corresponding Author: Dr. Sílvia Ferreira de Sousa, Department of Oral Surgery, Pathology and Clinical Dentistry, School of Dentistry, Federal University of Minas Gerais, Avenida Antonio Carlos, 6627, Pampulha, Belo Horizonte, Minas Gerais, Brazil. e-mail: silviafsousa@ufmg.br
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Introduction

Ectomesenchymal chondromyxoid tumor (EMCMT) is a rare benign soft tissue neoplasm affecting the oral cavity and was first described by Smith et al. in 1995 (Smith et al., 1995). Initially, EMCMT was reported within the "pleomorphic adenoma or myoepithelioma" category, and its definition was strictly based on its histopathological appearance (Smith et al., 1995). Even though some advances have been made, the current Word Health Organization (WHO) classification of head and neck tumors still defines EMCMT as a tumor of uncertain histogenesis (Muller and Tilakaratne, 2022). Genetic alterations, mainly *RREB1::MRTFB* translocations, have been identified in EMCMT, involving about 90% of cases (Dickson et al., 2018).

While it has become increasingly clear that EMCMT and myoepithelioma are distinct biological entities, some overlapping features still affect the precise diagnosis of these lesions (Smith et al., 2023). Furthermore, the rarity and the lack of strictly essential and desirable diagnostic criteria for EMCMT may contribute to a less distinct diagnosis in daily practice. Although narrative reviews have described the clinicopathological factors of EMCMT (Leeky et al., 2011; Kato et al., 2017; Truschnegg et al., 2018; Smith and Maynihan, 2023),

none of them systematically reviewed the histomolecular characterization of this entity nor addressed the association between clinicopathological features and genetic translocation in EMCMT of the oral and maxillofacial region. Therefore, it remains unclear whether the histopathological features of EMCMT are associated with the genetic background of the tumor. The objective of the present study was to perform a systematic review focusing on morphological findings and to evaluate the possible association between morphological features with the genetic background of the tumor.

Materials and methods

The reporting of this systematic review complies with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Page et al., 2021).

Eligibility criteria

Case reports or case series of EMCMT in the oral and maxillofacial region reported in English and with sufficient clinicopathological information to confirm the diagnosis were included. Exclusion criteria were articles in which the data could not be extracted, expert opinions/comments, and letters to the editor unless any of these types of articles provided sufficient and detailed data on EMCMT. Reports of a tumor with an *RREB1::MRTFB* fusion but without other histopathological features of EMCMT were also excluded. The diagnostic criteria were based on the updated WHO classification of head and neck tumors (Muller and Tilakaratne, 2022).

Information sources and search strategy

Electronic searches were conducted in the following databases: PubMed (National Library of Medicine), Web of Science (Clarivate Analytics), Scopus (Elsevier), Ovid (Wolters Kluwer), and Embase (Elsevier) in August 2023. Restriction on the date of publication was not imposed. The following search terms were used: (Ectomesenchymal chondromyxoid tumor) OR (Ectomesenchymal chondromyxoid tumour). Searches in Open Grey were limited to the first 300 hits (Haddaway et al., 2015), and hand searches in the reference list of the included articles were also carried out. The retrieved references were exported to EndNote software (Clarivate Analytics, Toronto, Canada) and duplicates were removed upon identification.

Study selection

The study selection was performed in two phases. The titles/abstracts of all references retrieved during the search were independently read by two review authors (RHJS and SFS). Articles whose titles/abstracts met the eligibility criteria were included immediately. When the abstract was unavailable or did not provide sufficient information, the full text was read and retrieved to assist the two authors in the decision to include or exclude. Possible disagreements between RHJS and SFS were resolved by means of discussion with a third author (FAF).

Data extraction

The following data were extracted from each article included: first author's last name, year of publication, continent and country where the case(s) was/were reported, number of reported cases, patient's age and sex, lesion size, evolution time, location, clinical aspects, diagnostic hypotheses, microscopic, immunohistochemical and molecular findings, recurrence (yes or no), and treatment.

Critical assessment of the included studies

A critical appraisal of the included articles was carried out by using the Joanna Briggs Institute (University of Adelaide) tool for case reports or series (Gagnier et al., 2013). The included articles were evaluated according to the following parameters: clear description of the demographic and microscopic characteristics of the individual with EMCMT, individual's history, current clinical condition, diagnostic method, treatment, clinical condition after intervention, adverse effects, and whether the clinical case provided a takeaway lesson. For each parameter, the included article was categorized as "yes" (good methodological quality), "no" (poor methodological quality), "unclear" or "not applicable".

Statistical analysis

Comparative analysis was performed with Statistical Package for the Social Sciences (SPSS) software, version 23.0 (SPSS Inc., Chicago, IL, USA), using the nonparametric Mann-Whitney test and a p-value <0.05 was considered statistically significant.

Results

Study selection

The electronic searches yielded 546 articles (PubMed: 137, Web of Science: 117, Embase: 175, Scopus: 61, and Ovid: 56) of which 53 studies reporting 114 individuals were included in this systematic review (Smith et al., 1995; Woo et al., 1995; Kannan et al., 1996; van der Wai and Van der Wall, 1996; Román et al., 1999; de Visscher et al., 2003; Ide et al., 2003; Kaplan et al., 2004; Bot et al., 2006; Goveas et al., 2006; Nigam et al., 2006; Seckin et al., 2008; Pires et al., 2009; Portnof et al., 2009; Angiero, 2010; Chopra et al., 2010; Nikitakis et al., 2010; Seo et al., 2010; Leeky et al.,

2011; Sengul et al., 2011; Gouvêa et al., 2012; Guzmán et al., 2012; Pak et al., 2012; Tsai et al., 2012; Closmann et al., 2013; Yoshioka et al., 2013; Cardin et al., 2014; Kale et al., 2014; Aldojain et al., 2015; Maraschin et al., 2015; Tajima and Koda, 2015; Argyris et al., 2016; Laco et al., 2016; Schep et al., 2016; Stecco et al., 2016; AlZamel et al., 2017; Kato et al., 2017; Almeida et al., 2018; Dickson et al., 2018; Sato et al., 2018; Truschnegg et al., 2018; Adorno-Farias et al., 2019; Ng et al., 2019; McNamara and Bloemena, 2019; Riju et al., 2019; Sakurai et al., 2020; Arteta et al., 2021; Bubola et al., 2021; Jung et al., 2021; Cunha et al., 2022; Naidoo et al., 2022; Agaimy et al., 2023; Smith et al., 2023). The included articles (Supplementary Material 1) were published between 1995 and 2023. Five were case series and 48 were case reports. Studies were conducted in five continents. A subset of the cases published by some references (Argyris et al., 2016; Schep et al., 2016) were molecularly analyzed by Dickson et al. (2018). In such cases, we have compiled data by gathering clinical, demographic, and morphological information from the original article.

Critical appraisal of the included studies

All 53 (100%) included articles provided a clear description of the patient's demographic characteristics and current clinical status. Forty-nine of the 53 (92.5%) provided information on patient history as a timeline. Diagnostic tests or assessment methods were reported in 100% of studies. Most articles provided intervention data (n=51; 96.2%) and information on the clinical condition after intervention (n=41; 77.4%) of affected individuals. Four (7.5%) reports identified adverse events. All articles (n=53; 100%) provided take-away lessons (Supplementary Material 2).

Demographic data and clinical features

Table 1 summarizes the clinicodemographic data, treatment performed, and outcomes of all tumors retrieved in this study, highlighting the molecularly confirmed tumors. Less than 10% of articles (5/53) analyzed this entity through FISH or RNAseq (Supplementary Material 1) by targeted or untargeted methods, and the diagnosis of EMCMT confirming the *RREB1::MRTFB* fusion was reported in 20/22 cases (91.0% of overall positivity). An *EWSR1* rearrangement was detected in four cases (17.4%) (Table 1).

For all cases retrieved, the mean age of affected individuals was 38.5 years (±17.4), and most were 50 to 59 years old (25.7%). Women (51.8%) were slightly more affected, with a female-to-male ratio of 1.07:1. Regarding location, 92% (105/114) of tumors were described in the tongue, with only two (2.0%) cases defined as intraosseous lesions, arising in the maxilla and mandible, both harboring an *RREB1::MRTFB* fusion. Nodular (n=52; 73.2%), sessile (n=18; 81.8 %), and nonulcerated (n=39; 83.0%) lesions were the most reported clinical aspects. The tumors were slow-growing (89.3%), measuring a mean of 15.5 mm in size, and had a mean time of evolution of 34.4 months. Eighty-one (71.0 %) tumors were treated with excisional biopsy, and local recurrence occurred in 7.6% (5/61) of cases. The mean time of follow-up was 35.0±47.7 months.

Considering the tumors with a diagnosis molecularly confirmed regarding *RREB1::MRTFB* or *EWSR1* status, independent of the genetic alteration, the clinico-demographic characteristics were similar to the all cases reported in the literature (Table 1).

Microscopic features of EMCMT in the literature

Tables 2 and 3 show the full information regarding the morphological and immunohistochemical characterization of EMCMT in the oral and maxillofacial region. Figure 1 illustrates the frequent histological and immunohistochemistry findings displayed by EMCMT. The main histopathological features of EMCMT described in the total of 114 cases were well-circumscribed (96/114; 84.2%), lobulated (76/114; 66.7%), reticulated (59/114; 51.8%), and arranged in sheets, cords, and strands (49/114; 42.9%). The presence of a myxoid stroma (101/114; 88.6%), chondroid (69/114; 60.5%), chondromyxoid stroma (65/114; 57.0%), and containing fibrous septae (49/114; 42.9%) were also tumor-outlined features. Approximately 74.0 % (84/114) of EMCMT lesions had spindle-shaped cells; round, ovoid, or epithelioid cells were mentioned in 89.5% (102/114). High incidences of eosinophilic to pale cytoplasm (36/114; 31.6%), pseudonuclear inclusions (47/114; 41.2%), pleomorphism (64/114; 56.1%) as well as muscle infiltration (40/114; 35.1%) were noted.

The immunohistochemistry features were reported in 81.6% (93/114) of cases. GFAP and S-100 were the most investigated markers. The following immunomarkers were more frequently positive: vimentin (33/33, 100.0% of overall positivity), cyclin D1 (4/4, 100.0%), GFAP (89/101, 88.1%), NSE (7/8, 87.5%), S-100 (83/96, 86.5%), CD56 (10/13, 76.9%), and CD57 (26/34, 76.5%) (Table 3).

Microscopic features of EMCMT molecularly investigated for RREB1::MRTFB fusions and/or EWSR1 re-arrangements

Table 4 summarizes the microscopic features of tumors molecularly investigated for *RREB1::MRTFB* fusions and *EWSR1* rearrangements. Microscopically, most of the EMCMT displaying an *RREB1::MRTFB* fusion were well-circumscribed (20/20), lobular (16/16) proliferations, separated by fibrous septae (13/14), exhibiting sheet-like (13/14), and reticular formations (14/15), in a myxoid background (19/20). Additionally, chondroid features in the stroma were reported in seven cases (7/7).

Within the four *EWSR1* rearrangement cases, all (4/4) were also well-circumscribed, three (3/3) lobular

Table 1. Summary of the clinical characteristics of ectomesenchymal chondromyxoid tumors of the oral and maxillofacial region, comparing cases retrieved from the literature and cases with molecular alterations.

Variable		All cases retrieved (n=114) n (%)	Cases positive for *RREB1::MRTFB* fusions (n=20†/22) n	Cases positive for *EWSR1* rearrangements (n=4/23¥) n
Continent	North America	71 (63.2)	19	4
	South America	11 (9.6)	0	0
	Asia	21 (18.4)	0	0
	Europe	10 (8.8)	1	0
	Africa	1 (1)	0	0
Ethnicity	White	19 (67.9)	NA	NA
	No white	8 (32.1)	NA	NA
Age (Years/ decades of life)		Mean: 38.5±17.4 Range: 2-78	Mean: 37.8±13.96 Range: 13-59	Mean: 38.75±12.8 Range: 22-54
	0-9	7 (6.4)	0	0
	10-19	8 (7.3)	3	0
	20-29	22 (20.2)	2	1
	30-39	19 (17.4)	6	1
	40-49	16 (14.7)	2	1
	50-59	28 (25.7)	7	1
	60-69	5 (4.6)	0	0
	70-79	4 (3.7)	0	0
Sex	Female	59 (51.8)	13	3
	Male	55 (48.2)	7	1
	Ratio	1.07:1	1.85:1	3:1
Anatomical location	Tongue	105 (92.0)	18	4
	Palate	4 (3.0)	0	0
	Buccal mucosa	1 (1.0)	0	0
	Gingiva	1 (1.0)	0	0
	Tonsilla	1 (1.0)	0	0
	Mandible	1 (1.0)	1	0
	Maxilla	1 (1.0)	1	0
Clinical presentation	Nodule	52 (73.2)	NA	NA
	Tumor	18 (25.4)	2	NA
	Polyp	1 (1.4)	NA	NA
Submucosal mass	Yes	30 (96.8)	NA	NA
	No	1 (3.2)	NA	NA
Implantation	Sessile	18 (81.8)	1	NA
	Pediculated	4 (18.2)	NA	NA
Color of the lesion	Normal mucosa	26 (74.3)	1	NA
	Other colors	9 (25.7)	NA	NA
Surface	Nonulcerated	39 (83.0)	NA	NA
	Ulcerated	8 (17.0)	1	NA
Consistency	Firm	32 (74.4)	1	NA
	Soft	10 (23.3)	1	NA
	Rubbery	1 (2.3)	NA	NA
Growing	Slow	25 (89.3)	2	NA
	Fast	1 (3.6)	NA	NA
	Slow to fast	2 (7.1)	NA	NA
Symptomatology	No	52 (88.1)	2	NA
	Yes	7 (11.9)	NA	NA
Size of the lesion (mm)	Mean (SD)	15.5±10.4	15.5±7.8	NA
	Range	3-57	7-30	NA
Time of evolution (months)	Mean (SD)	34.4±53.0	15.46±7.78	NA
	Range	0-240	7-30	NA
Treatment	Excisional biopsy	81 (71.0)	9	1
Recurrence	No	61 (92.4)	7	1
	Yes	5 (7.6)	1	NA
Follow-up (months)	mean ±SD	35.0±47.7	38.0±35.5	NA
	Range	0-240	0.2-102	NA

NA, Information not available. (†) This consists of 18 cases from Dickson et al. (2018), one case from Bubola et al. (2021), and one from Agaymi et al. (2023). Of the 19 positive cases reported by Dickson, case #12 was excluded due to divergences displayed in the text and correspondent table. (¥) This consists of 11 cases reported by Argyris et al. (2016), cases #1 to #11 by Dickson et al. (2018), and case #3 by Agaymi et al. (2023). RNA Seq performed by Dickson et al (2018) and Agaymi et al. (2023) used the same gene fusion panel including the *RREB1::MRTFB* and *EWSR1* genes. However, the panel used in RNA seq performed by Bubola et al. (2016) was not mentioned.

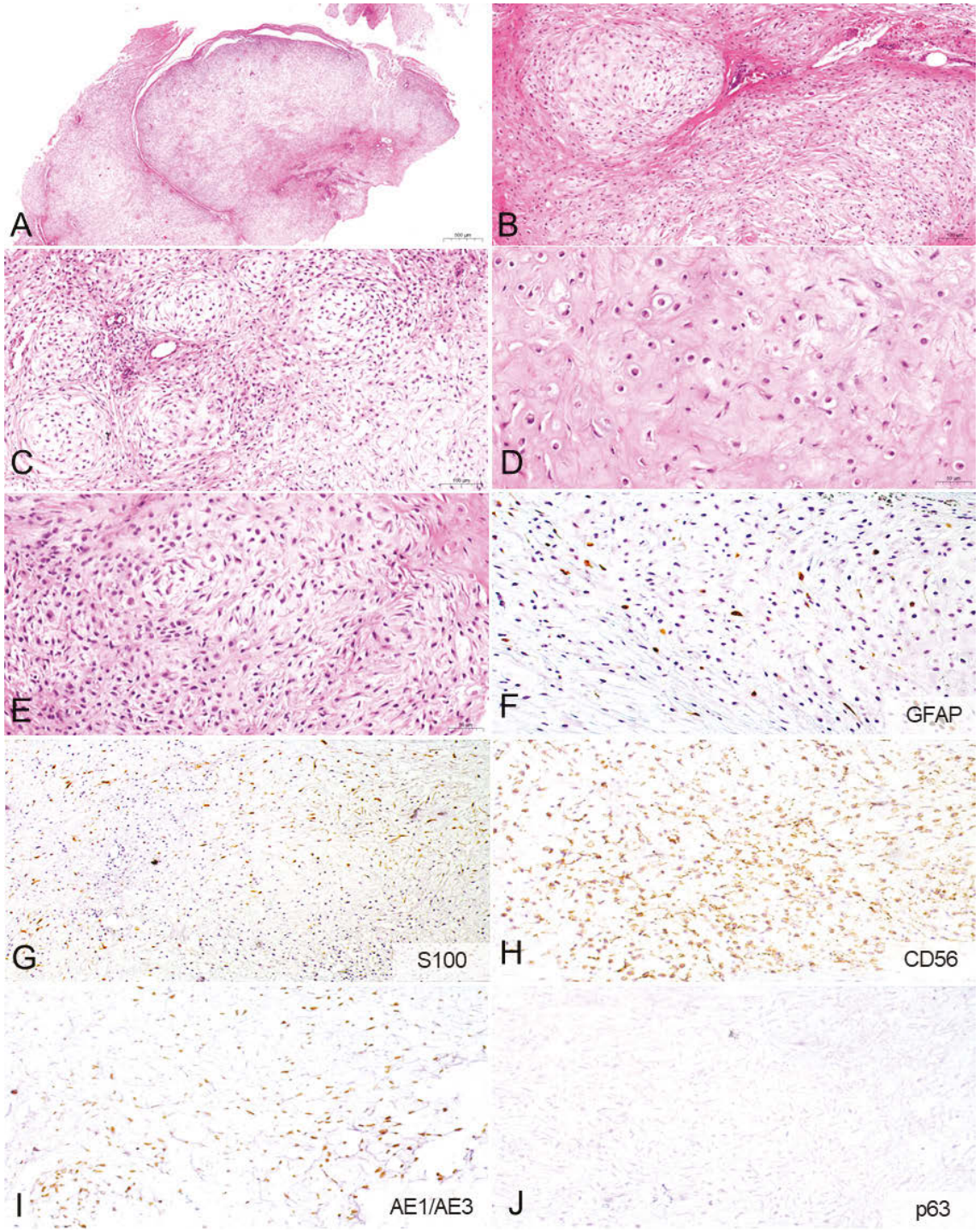

Fig. 1. Histopathological and immunohistochemical features of ectomesenchymal chondromyxoid tumor harboring an *RREB1::MRTFB* fusion affecting the floor of the mouth. **A.** histopathological analysis exhibits a well-defined, partly encapsulated benign lesion, interspersed with more and less collagenized areas (hematoxylin and eosin [H&E]). **B.** The lesion is organized into concentric lobules interposed by dense connective tissue septa in some areas (H&E). **C, D.** Inside the lesion, the tumor cells are oval, round, or spindle-shaped, with uniform oval nuclei, arranged in a myxoid and chondroid matrix (H&E). **E.** The presence of plasmacytoid cells, binucleated cells, and hyperchromatic cells could also be detected (H&E). **F-J.** The neoplastic cells demonstrate focal positivity for glial fibrillary acidic protein (GFAP) **(F)** diffuse positivity for S100 **(G)** and CD56 **(H)**, besides focal positivity for AE1/AE3 **(I)** and negativity for p63 **(J)**. 3,3′-diaminobenzidine [DAB] F, H, × 200; G, I, J, × 100.

and non-encapsulated proliferations, showing fibrous septae (2/2), immersed in a myxoid (2/3), chondroid (2/2) or chondromyxoid (1/2) stroma. No significant association between these morphologic characteristics and *RREB1::MRTFB* status was seen (Table 4).

Regarding cell morphology, the presence of spindle cells (18/18), followed by polygonal cells (13/13), was the main phenotype in tumors harboring *RREB1::MRTFB* fusions. Tumors with *EWSR1* rearrangements were predominantly composed of spindle cells (3/3) and round/ovoid cells (3/3), with an amphophilic cytoplasm (2/2). In *RREB1::MRTFB* tumors, besides cells exhibiting an amphophilic cytoplasm (14/14), the cells frequently showed multilobulated nuclei (13/13), with pseudonuclear inclusions (13/13). Pleomorphism was highly present in *RREB1::MRTFB* tumors (18/19), and in EWSR1-positive cases (4/4); however, mitotic figures were scarce in all tumors displaying genetic alterations. A more infiltrative growth pattern with muscle entrapment was observed in 82.4% (14/17) of cases with an *RREB1::MRTFB* fusion. Contrarily, the presence of muscle infiltration was only present in one (1/4) *EWSR1*-positive tumor case; and this infiltration was significantly higher (15/18) in *EWSR1*-negative tumors ($p<0.05$) (Table 4).

No significant association between immunohistochemical characteristics and genetic status was seen (Table 4). Moreover, no significant differences were observed between tumors showing *RREB1::MRTFB* alterations and tumors featuring EWSR1. Although not statistically significant, the immunoexpression for S-100 was consistently positive (17/17) in *RREB1::MRTFB*-positive and *EWSR1*-negative (12/12) tumors (p=0.07). *RREB1::MRTFB* positivity was also mainly accompanied by positivity for GFAP (17/18), SMA (9/11), desmin (10/14); and by high negativity for myogenin (12/14), and SOX-10 (6/6) in all cases tested (Table 4).

Table 2. Frequency of histopathological features in ectomesenchymal chondromyxoid tumors of the oral and maxillofacial region retrieved from the literature.

Histopathological characteristic	n (%)
Encapsulated	
Yes	3 (6.1)
No	46 (93.9)
Growth pattern	
Well-circumscribed	96 (31.5)
Lobular	76 (25.0)
Net-like /reticular	59 (19.3)
Sheets, cords, and strands	49 (16.1)
Storiform or fascicular	4 (1.3)
Papillary	1 (0.32)
Slit-like	19 (6.3)
Stroma, n=318*	
Fibrous septae	49 (15.4)
Myxoid	101 (31.8)
Chondroid	69 (21.7)
Chondromyxoid	65 (20.4)
Hyalinized	32 (10.1)
Mucinoid	2 (0.6)
Cell morphology, n=262*	
Spindle	84 (32.1)
Round	60 (22.9)
Ovoid	36 (13.7)
Epithelioid	6 (2.3)
Polygonal	56 (21.4)
Stellate	8 (3.1)
Multinucleated	12 (4.5)
Cytoplasm, n=47*	
Eosinophilic to pale	36 (76.6)
Intracytoplasmic vacuoles	11 (23.4)
Nucleus, n=142*	
Lobulated	17 (11.9)
Pseudonuclear inclusions	47 (33.1)
Pleomorphism	64 (45.1)
Mitosis	14 (9.9)
Extension into adjacent structures, n=72*	
Pushing border	26 (36.1)
Muscle infiltration	40 (55.5)
Neural infiltration	3 (4.2)
Adipose infiltration	3 (4.2)
Other findings, n=32*	
Osseous component	2 (6.3)
Calcifications	6 (18.7)
Hemorrhage	23 (71.9)
Necrosis	1 (3.1)

* This variable was not analyzed by the number of individuals but by the number of features presented. Individualized data can be seen in Supplementary Material 1.

Table 3. Immunohistochemistry characterization of ectomesenchymal chondromyxoid tumors of the oral and maxillofacial region retrieved from the literature. Number of immunopositive cases in relation to the total of cases investigated.

Immunohistochemistry marker	Positive Cases n (total investigated)
Vimentin	33 (33)
GFAP	89 (101)
S100	83 (96)
CD56	10 (13)
CD57	26 (34)
SMA	41 (74)
NSE	7 (8)
SMMHC	5 (7)
Cyclin D1	4 (4)
Desmin	18 (47)
AE1/AE3	23 (64)
CD10	3 (4)
Synaptophysin	3 (7)
p63	7 (27)
D2-40	2 (4)
EMA	3 (23)
HHF35	1 (4)
Myogenin	2 (19)
Calponin	1 (10)
SOX-10	1 (11)
CD34	0 (12)
Ck7	0 (6)
Ck8	0 (7)
Ck18	0 (5)
Ck20	0 (4)
HMB45	0 (4)

Table 4. Association between microscopic characteristics and molecular data in ectomesenchymal chondromyxoid tumors of the oral and maxillofacial region that were screened for fusions between *RREB1::MRTFB* or rearrangements in *EWSR1*.

Variable		*RREB1::MRTFB* fusion			*EWSR1* rearrangements		
		No n=2	Yes n=20	*p*	No n=23	Yes n=4	*p*
Encapsulated	No	NA	0	-	9	3	-
	Yes	NA	5		NA	NA	
Circumscribed	No	0	0	-	NA	NA	-
	Yes	2	20		19	4	
Lobular	No	0	0	-	NA	NA	-
	Yes	2	16		11	3	
Sheets, cords, or strands	No	0	1	*1.000*	NA	NA	-
	Yes	2	13		10	1	
Netlike/reticular	No	0	1	*1.000*	NA	NA	-
	Yes	2	14		10	1	
Fibrous septa	No	0	1	*1.000*	NA	NA	-
	Yes	2	13		11	2	
Myxoid stroma	No	0	1	*1.000*	2	1	*0.371*
	Yes	2	19		17	2	
Chondroid stroma	No	NA	0	-	1	0	*1.000*
	Yes	NA	7		9	2	
Chondromyxoid stroma	No	NA	1	-	2	1	*0.455*
	Yes	NA	6		8	1	
Spindle cells	No	NA	NA	-	NA	NA	-
	Yes	2	18		13	3	
Polygonal cells	No	NA	NA	-	NA	NA	-
	Yes	2	13		10	1	
Round/Ovoid cells	No	NA	0	-	NA	NA	-
	Yes	NA	6		6	3	
Nuclear multilobulation	No	NA	NA	-	NA	NA	-
	Yes	2	13		10	1	
Pseudonuclear inclusion	No	NA	NA	-	NA	NA	-
	Yes	2	13		10	1	
Amphophilic cytoplasm	No	NA	NA	-	NA	NA	-
	Yes	2	14		10	2	
Pleomorphism	No	0	1	*1.000*	2	0	*1.000*
	Yes	2	18		16	4	
Mitosis	No	2	17	*1.000*	18	3	*0.324*
	Yes	0	2		1	1	
Muscle entrapment	No	0	3	*1.000*	3	3	*0.046**
	Yes	2	14		15	1	
S-100	Negative	1	0	*0.105*	0	1	*0.077*
	Positive	1	17		12	0	
GFAP	Negative	1	1	*0.195*	1	1	*0.275*
	Positive	1	17		11	11	
AE1/AE3	Negative	1	11	*1.000*	8	2	*0.524*
	Positive	1	8		5	0	
Desmin	Negative	2	4	*0.125*	4	1	*1.000*
	Positive	0	10		5	0	
Myogenin	Negative	2	12	*1.000*	7	2	*1.000*
	Positive	0	2		1	0	
SMA	Negative	1	2	*0.423*	1	1	*0.222*
	Positive	1	9		7	0	
SOX-10	Negative	2	6	-	6	1	-
	Positive	0	0		0	0	
RREB1::MRTFB	No	-	-	-	1	1	*0.242*
	Yes	-	-		13	1	

NA, Information not available. Individualized data can be seen in Supplementary Material 1. Chi-Square test. (*) *p*-value<0.05 was considered statistically significant.

The *EWSR1*-positive tumors showed immunopositivity for GFAP (1/2) and CD56 (1/1). Considering *EWSR1*-negative tumors, they also showed consistent negativity for myogenin (7/8) and SOX-10 (6/6), and a high positivity for SMA (7/8). The results for AE1AE3 showed a more heterogeneous immunoexpression, with 11/18 of *RREB1::MRTFB* tumors and 8/13 of *EWSR1*-negative tumors showing negativity for this marker. Other scarce microscopic features, present in only one or two cases or lacking available information, can be accessed online in Supplementary Material 1.

Discussion

Diagnosing EMCMT can be challenging due to its overlapping features with other mesenchymal and salivary gland tumors and its rarity, with slightly more than 100 cases reported in the literature, as demonstrated in this review. Over the past decade, next-generation sequencing has revealed significant fusion genes in oral tumors, especially in mesenchymal soft tissue neoplasms (Gomes et al., 2023) and in salivary gland tumors (Skálová et al., 2022). Although most fusions have mainly diagnostic purposes, some are associated with clinical behavior and morphological aspects of the lesion, highlighting that the genetic signature might be predictive of certain tumor features (Almagro et al., 2022; Skálová et al., 2022). In this study, we systematically summarized the scientific literature focusing on histomolecular findings and evaluated whether morphologic features were associated with the genetic background of EMCMT. The morphological characteristics found in this study that most represented EMCMT were: (i) EMCMT characteristically occurs in the deep lamina propria without connection to the overlying epithelium, even though pushing borders were reported in some cases; (ii) a demarcated lesion, but not encapsulated, lobulated, reticulated with sheets, cords, and strands in a myxoid and chondroid stroma; and (iii) it comprises variable cytomorphological cells (mostly round and spindle) with eosinophilic to amphophilic cytoplasm that may contain nuclei with lobulation and pseudoinclusions. Overall, we found that the presence of an *RREB1::MRTFB* fusion or chromosome alterations in the *EWSR1* gene were not highly specific to the morphological features of EMCMT.

The *RREB1::MRTFB* fusion was detected in 91% of the investigated cases, consisting of tumors localized almost exclusively to the tongue (90.0%), the two remaining cases (10.0%) being intraosseous lesions (Agaimy et al., 2023; Bubola et al., 2021). As extra-glossal EMCMT is rare (Nigam et al., 2006; Gouvêa et al., 2012; Stecco et al., 2016; Truschnegg et al., 2018; Ng et al., 2019; Bubola et al., 2021; Agaimy et al., 2023; Maraschin et al., 2025), it is unknown if the presence and frequency of the *RREB1::MRTFB* fusion are shared by EMCMT arising in soft tissues of the oral cavity outside the tongue. This fusion has already been identified in EMCMT affecting sites beyond the head and neck region (Makise et al., 2020; Agaimy et al., 2023), suggesting that this fusion may be tightly linked to the tumor regardless of anatomical site.

In the present study, although *RREB1::MRTFB* represents the leading molecular alteration, alternative rearrangements involving *EWSR1* in EMCMT were also identified in four cases (14.3%). Interestingly, Dickson et al. (2018) found that cases harboring *EWSR1::CREM* or that were negative for *EWSR1* or *RREB1::MRTFB* fusions lacked the typical architectural morphology of EMCMT, suggesting that some histopathological characteristics could be fusion-type specific. In the current study, tumors lacking *EWSR1* rearrangements were associated with the presence of muscle infiltration, with 83.3% of *EWSR1*-negative tumors presenting this feature. The *RREB1::MRTFB* status of these published EWSR1-negative tumors is unknown. However, considering that *RREB1::MRTFB* is a hallmark in 90% of EMCMT, we can speculate that most of the *EWSR1*-negative tumors might be *RREB1::MRTFB* positive and could have muscle infiltration as a distinct feature.

Considering the presence of high-risk parameters, such as pleomorphism, mitosis, and tumor necrosis, pleomorphism was observed in 56% of tumors, whereas mitosis was rare, occurring in less than 10% of cases and none were directly related to an *RREB1::MRTFB* fusion or chromosome alterations in *EWSR1*. This result highlighted that EMCMT has mild to moderate morphological features, reflecting an indolent clinical course as demonstrated in the present review despite the relatively short clinical follow-up reported in some cases. Furthermore, the occurrence of one fusion does not inherently indicate more aggressive behavior, in some cases being related to low and intermediate-grade tumors as seen in salivary carcinomas (Skálová et al., 2022). Genetic background did not appear to impact the other microscopic findings of EMCMT.

Although the histogenesis of EMCMT is still uncertain (Muller and Tilakaratne, 2022), the immunophenotype results indicated neural markers with high positivity in EMCMTs retrieved from the literature. However, we did not find an association between staining that labels cells of differing lineages with the genetic background of the tumor. A notable point is the consistent lack of SOX-10 expression in EMCMT regardless of the genetic status of *RREB1::MRTFB* fusion or chromosome alterations in *EWSR1*. SOX-10, a transcription factor belonging to the SOX (SRY-related HMG-box) family of proteins, related to the development and maintenance of neural crest-derived tissues, is also expressed in myoepithelial cells from salivary glands, being detected in myoepitheliomas derived from salivary glands and skin (Naujokas et al., 2014). Consequently, despite the similar morphologies of EMCMT and salivary gland myoepitheliomas, SOX-10 and additional myoepithelial markers such as calponin and p63 may offer a valuable tool for distinguishing between these tumors. The variability in positivity for AE1/AE3, desmin, and SMA reflects the

considerable variation in immunohistochemical staining patterns of EMCMT.

Considering that cases with molecularly validated diagnoses in the literature represent only about 20% of the total published cases, more publications containing molecular characterization could expand our knowledge of the clinicopathological characteristics and genetics of this tumor. The background genetic signature analyses were performed with different platforms. The use of high throughput sequencing in more cases of EMCMT could highlight whether other driver alterations coexist with the already identified hallmark.

In conclusion, our study shows that the presence of the fusion *RREB1::MRTFB* or chromosome alterations in the *EWSR1* gene was not highly specific to the morphological features of EMCMT. This warrants further investigation regarding whether different genetic alterations translate into histologic and/or phenotypic differences in EMCMT. The summarized histopathological and immunohistochemical characteristics contribute to a more precise microscopic diagnosis of EMCMT.

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