Mucoepidermoid Carcinoma: A Comparison of Histologic Grading Systems and Relationship to *MAML2* Rearrangement and Prognosis

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Abstract

Mucoepidermoid carcinoma (MEC) is the most common salivary gland malignancy, but categorization is complicated by variability in grading systems and uncertain prognostic significance of *MAML2* rearrangement. The aims of this study were to determine the prognostic significance of MEC grading systems and *MAML2* rearrangement status. Fifty-three carcinomas

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originally diagnosed as MEC (45 primary; 8 recurrent) of major and minor salivary glands were graded according to modified Healey, Brandwein, AFIP, and Katabi systems. Fluorescence in-situ hybridization for *MAML2* rearrangement was performed. Clinical features and outcomes were recorded. Twenty-five (47%) carcinomas scored the same in all grading systems. The most common histologic feature leading to a diagnosis of intermediate grade was isolated solid growth. Brandwein assigned the highest percentage of high grade (29%) and AFIP the highest percentage of low grade (80%). *MAML2* was rearranged in 37/46 (80%) cases. Forty-three (81%) were morphologically compatible with MEC, and these were more likely to be low-intermediate grade and *MAML2*-rearranged. Of primary carcinomas, 6 (13%) recurred. Statistically significant univariate risk factors for recurrence included non-MEC morphology, stage T4, and high Brandwein grade. Margin status, *MAML2* rearrangement, and isolated solid growth were not predictive of recurrence. A binary grading system (Brandwein high versus low-plus-intermediate) could be considered to better reflect biologic behavior in MEC. Our study confirms that *MAML2* wildtype tumors more likely represent high grade non-MECs, and prior studies demonstrating worse prognosis in *MAML2*-non-rearranged MECs may be diluted by high grade non-MECs.

Keywords

Salivary gland cancer; mucoepidermoid carcinoma; grade; *MAML2*; prognosis; recurrence

Introduction:

Salivary gland carcinomas represent approximately 11% of head and neck malignancies, the most common being mucoepidermoid carcinoma (MEC).[1,2] Variety in clinical presentation, behavior, and histologic appearance has made these carcinomas challenging to stage and treat. In the initial description of "mucoepidermoid tumors," Stewart et al. separated them into benign and malignant.[3] Since then, all tumors have been determined to possess malignant potential[4] (hence the unifying term mucoepidermoid carcinoma), and multiple histopathologic grading systems have been created in order to better stratify patients for prognostication and treatment, namely the modified Healey[5,6], AFIP[7,8], Brandwein[9], and Katabi[10]. All grading systems (whether quantitative or qualitative) have three grades: low, intermediate, and high. One difficulty lies in the inconsistency of the grading systems: applying the different grading systems to the same tumor may result in different grades, with the Brandwein system tending to assign higher grades and the AFIP system lower grades. Furthermore, treatment for a histologically intermediate grade carcinoma is not straightforward. Low grade tumors generally require complete surgical excision and high grade tumors are treated with adjuvant radiotherapy with or without chemotherapy.[11] Intermediate grade carcinomas may behave similarly to low grade carcinomas, and treatment with surgical excision alone has been considered.[12,13] Lastly, presence of a *CRTC1/3-MAML2* fusion has been suggested by some to correlate with lower histologic grade and to portend better prognosis.[14–22] However, these results have not been uniformly reproducible, and *MAML2* rearrangement can be present in high grade or high stage MEC and may not necessarily correlate to prognosis.[23–29] Arguably, fusion-negative tumors may represent other high grade non-mucoepidermoid carcinomas.[16,24,25,28,30] Clarification of the divergent grading systems, the significance of *MAML2* rearrangement status, and the possible

treatment protocols for intermediate grade tumors is warranted. The aims of this study are to compare mucoepidermoid carcinoma grading systems and to evaluate *MAML2* rearrangement status in the context of patient outcomes in order to determine the prognostic significance of histologic grading systems and genetics.

Materials & Methods:

With Institutional Review Board approval, cases of primary head & neck mucoepidermoid carcinoma were identified in the diagnostic surgical pathology database, between years 1993 and 2018. Resection specimens of primary MECs of major and minor salivary glands were included. MECs occurring outside the head & neck (including trachea, lung, and breast) were excluded. Diagnostic hematoxylin and eosin (H&E) slides and formalin-fixed paraffin embedded (FFPE) blocks were retrieved from the diagnostic surgical pathology archives.

Histologic Review and Grading:

Diagnostic H&E slides were reviewed by two head & neck pathologists (NAC, ML) and each carcinoma was graded according to four published grading systems: 1) the qualitative *modified Healey* system of Batsakis and Luna[5,6]; 2) the quantitative *AFIP* system of Auclair, Goode, and Ellis[7,8]; 3) the quantitative system of *Brandwein*, et al[9]; and 4) the qualitative system of *Katabi*, et al[10]. [Table 1] The grade assigned by the original signout pathologist was recorded. Over the 25 year time period, the original signout pathologist varied. Whether the tumor was morphologically compatible with mucoepidermoid carcinoma (as determined by head & neck pathologists NAC & ML) was also recorded.

MAML2 Rearrangement:

For all cases with available FFPE blocks, a tumor-rich block was selected. Fluorescence in-situ hybridization (FISH) for *MAML2* was performed on paraffin-embedded tissue sections using locus specific, dual-color break apart probes consisting of a 680 Kb fragment corresponding to the 5' *MAML2* gene labelled in ZyGreen and a 370 Kb fragment corresponding to the 3' *MAML2* gene labelled in ZyOrange and (Zytovision, Bremerhaven, Germany). Briefly, slides were deparaffinized in CitriSolv (Decon Laboratories Inc, Kings of Prussia, PA) three times for 10 minutes each followed by two dehydration steps using 100% ethanol alcohol for 5 minutes each. Tissue sections were immediately transferred to 0.2N Hydrochloric acid (Thermo Fisher Scientific, Waltham, MA) for 30 minutes at room temperature followed by Sodium Thiocyanate (Sigma-Aldrich, St Louis, MO) for 30 minutes at 80°C. Tissue sections were digested in protease solution (Sigma-Aldrich, St Louis, MO) for six minutes followed by 2x SSC (Invitrogen, Carlsbad, CA), fixed in 10% buffered formalin for 10 minutes followed by 2x SSC twice for 5 minutes. Tissue sections were manually denatured at 80°C for 5 minutes in 70% Formamide (Thermo Fisher Scientific, Waltham, MA) followed by sequentially dehydrated in ethanol alcohol (70%, 85%, and 100%) and air-dried at room temperature. After applying probe, the slides were hybridized overnight in a humidified chamber at 37°C. Tissue sections were washed the next day in 0.3% NP40/2× SSC at 75°C for 2 minutes followed by 0.3% NP40/2× SSC at room temperature. Slides were mounted in Vectashield mounting medium (Vector Laboratories, Burlingame, CA) with 3 uL of DAPI 4′,6-diamidino-2-phenylindole (Sigma-Aldrich, St

Louis, MO). A total of 50 tumor nuclei were manually evaluated for each case. FISH results were considered positive for a *MAML2* rearrangement if >10% of tumor cells showed a separation of the orange and green signals by 2 signal widths, or if a deletion of the green signal was noted.

Clinical Outcomes & Statistical Analysis:

Patient data was collected from the institution's electronic medical record. Data included: age, sex, date of primary surgical resection, site of primary tumor, size of primary tumor, margin status, pathologic stage, and treatment. Outcomes were recorded as date and site of recurrence (including locoregional versus distant recurrence) and date and status at last follow-up. Kaplan-Meier estimates of survival were compared using log-rank tests. Tests of concordance between categorical variables were performed using Fisher's exact test. Statistical programming was completed in R (version 3.4.0), using packages "survival", "ggplot2", and "survminer".

Results:

A total of 53 resection specimens with an original diagnosis of mucoepidermoid carcinoma from major and minor salivary glands of the head & neck were identified and present in the archives. Forty-two were primary resections and 3 were re-excisions for positive margins (of which primary resection was performed at an outside institution and slides were reviewed internally). As the re-excisions represent part of the primary surgical treatment of these patients, they will be considered along with the initial primary resections for a total of 45 primary resections. Eight were recurrences (of which primary resection was performed at an outside institution, slides were not available, and recurrence occurred 6–134 months following reported primary resection). The most frequent primary site was minor salivary gland (n=32: 14 palate, 5 tongue, 3 retromolar trigone, 2 oropharynx, 2 floor of mouth, 2 mandible, 2 buccal, 1 lip, and 1 external ear canal) followed by parotid (n=20) and submandibular gland (n=1). Average age of patients with primary carcinomas was 52.7 (range 9–85) and that of patients with recurrent carcinomas 49.6 (range 28–69). Overall, females (n=34) were more frequently affected than males (n=19). Average size of the primary tumors was 1.8 cm (range 0.3–6.8 cm). Of patients with primary tumors, pathologic stage was T1 in 28 patients (18 NX, 9 N0, 1 N2), T2 NX in 1 patient, T3 in 4 patients (3 N0, 1 N2b), T4a in 9 patients (4 NX, 4 N0, 1 N1), and TX in 3 patients (2 NX, 1 N2). Surgical margins of primary tumors were negative in 29 patients and positive in 16 (6 in which the separately submitted margins were negative). Ten primary patients received adjuvant radiotherapy with or without chemoradiotherapy: 4 patients with T4 disease (3 N0, 1 N1), 2 patients with T3 disease (1 N0, 1 N2b), 1 patient with N2 disease, 2 patients with intermediate to high grade disease and close or positive margins, and 1 patient with low grade disease and positive margins. [Table 2, Supplementary Figure 1]

Histologic Review and Grading:

Upon contemporary review and re-grading of all cases, 25 (47%) scored the same in all grading systems (15 low and 10 high); the remaining 28 (53%) scored differently in in at least 2 systems.

Of the 45 primary resections/re-excisions, the original signout grade was high in 5 cases, intermediate in 12, and low in 28. Upon re-review, 7 scored high grade in all 4 systems, 6 scored high grade in 1–2 systems, 18 scored at most intermediate grade in at least 1 system, and 14 scored low grade in all systems [Table 2]. Specifically, carcinomas scored as follows: using the Brandwein system, 13 (29%), 18 (40%), and 14 (31%) were high, intermediate, and low grade, respectively; using the Katabi system, 8 (18%), 15 (33%), and 22 (49%) were high, intermediate, and low grade, respectively; using the modified Healey system, 8 (18%), 10 (22%), and 27 (60%) were high, intermediate, and low grade, respectively; using the AFIP system, 7 (16%), 2 (4%), and 36 (80%) were high, intermediate, and low grade, respectively [Figure 1]. The distribution was similar when considering only the 40 primary cases that were morphologically compatible with MEC on contemporary re-review [Supplementary Figure 2].

Forty percent (18/45) of primary carcinomas scored at most intermediate grade in at least one system. The most frequent histologic finding resulting in upgrade from low to intermediate grade was solid growth (11, 61%), followed by peripheral growth in nests and islands (4, 22%), and bony invasion (3, 17%) in palatal and mandibular tumors. Histologic features present only in the 13 tumors scoring high grade in at least one system included: anaplasia (7, 54%), necrosis (6, 46%), increased mitoses (5, 38%), perineural invasion (4, 31%), and lymphovascular invasion (2, 15%). Additionally, peripheral growth in nests and islands was present in 11 (85%), solid growth in 11 (85%), and bony invasion in 3 (23%).

Of the 8 recurrences, the original signout grade was high in 3 cases, intermediate in 3, and not specified in 2. Upon re-review, three scored high grade in all 4 systems, 4 scored high grade in 1–2 systems, and 1 scored low grade in all systems (primary ear canal carcinoma) [Table 2]. Specifically, carcinomas scored as follows: using the Brandwein system, 7 (88%) and 1 (13%) were high and low grade, respectively; using the Katabi and modified Healey systems, 5 (63%), 2 (25%), and 1 (13%) were high, intermediate, and low grade, respectively; using the AFIP system, 3 (38%), 3 (38%), and 2 (25%) were high, intermediate, and low grade, respectively.

After histologic review, 43 (81%) cases were morphologically compatible with mucoepidermoid carcinoma [Figure 2]. Forty were primary carcinomas and 3 were recurrences. Ten (19%) cases were not morphologically compatible with MEC [Figure 3]. Five were primary carcinomas and 5 were recurrences. Of all 20 carcinomas scoring high grade in Brandwein, 11 (55%) were morphologically MEC; of all 33 carcinomas scoring low-intermediate grade in all systems, 32 (97%) were morphologically MEC (p=0.0003) [Table 3A]. Of the 13 primary carcinomas scoring high grade in Brandwein, 9 (69%) were morphologically MEC; of the 32 primary carcinomas scoring low-intermediate grade in all systems, 31 (97%) were morphologically MEC (p=0.0198) [Table 3B]. Of 42 primary cases with known pathologic T stage, 38 (90%) were morphologically MEC, which was not different among patients with T1-T3 (30/33, 91%) compared to T4 (8/9, 89%) disease (p=0.6344) [Table 3C].

MAML2 Rearrangement:

FISH was attempted on all 53 cases. Hybridization failed in 7, including 3 due to decalcification. Of the 46 successful cases, 37 (80%) were positive for *MAML2* rearrangement and 9 (20%) were negative (2 of which showed polysomy). The positive cases included 3 clinically recurrent cases in which the primary was not reviewed; the remaining 34 positive cases were primary resections. All 37 positive cases had >48% rearranged cells (mean 90%, median 90%, range 48–100%). Two cases had <10% rearranged cells (2% and 4%); these cases were considered negative. One primary, high grade, morphologically MEC case had an unusual FISH pattern: 90% of cells had 2–3 single orange signals (3' of the *MAML2* gene), but no single green signals (5' of the *MAML2* gene). This pattern could represent an unbalanced rearrangement, gain of the region 3' to *MAML2*, or deletion of the region 5' to *MAML2*. Because it was suspicious for rearrangement, it was considered positive for the purposes of this study.

Of the 7 unsuccessful cases, 3 were morphologically compatible with mucoepidermoid carcinoma. Of the 37 fusion-positive and 9 negative cases, 37 (100%) and 3 (33%), respectively, were morphologically compatible with mucoepidermoid carcinoma (p=0.000008) [Table 3D]. Of the 37 fusion-positive and 9 negative cases, 9 (24%) and 7 (78%), respectively, were Brandwein high grade (p=0.0049) [Table 3E]. In other words, 93% (28/30) Brandwein low-intermediate grade were *MAML2*-rearranged, and 56% (9/16) Brandwein high grade were *MAML2*-rearranged. Considering only the primary cases morphologically compatible with MEC: of the 34 positive and 3 negative cases, 7 (21%) and 1 (33%), respectively, were Brandwein high grade (p=0.5297) [Table 3F]. Of primary cases with known pathologic T stage, 86% (32/37) were *MAML2*-rearranged, which was not different among patients with T1-T3 (27/31, 87%) compared to T4 (5/6, 83%) disease (p=0.6102) [Table 3G]. When considering only the primary cases morphologically compatible with MEC, *MAML2*-rearrangement was also not different among patients with T1-T3 (27/29, 93%) compared to T4 (5/6, 83%) disease (p=0.4417) [Table 3H].

Clinical Outcomes:

Of the 45 patients with primary resections/re-excisions, 6 (13%) recurred: patient 1 had a left parotid pT4 N0 carcinoma (morphologically MEC; high grade in all systems) who received adjuvant chemoradiation and recurred in the left submandibular area 9 months after resection; patient 2 had a palate T4 NX carcinoma (morphologically not MEC; high grade in Brandwein, low grade in AFIP, intermediate in Katabi and modified Healey) who was treated with surgery and had a rib metastasis 2 years after resection; patient 3 had a parotid T4 N1 carcinoma (morphologically MEC; high grade in Brandwein and Katabi, intermediate in modified Healey and AFIP) who received adjuvant chemoradiation, had a chest wall metastasis 1 year after resection, and died of diffuse metastasis 2.4 years after resection; patient 4 had a TX NX buccal carcinoma (morphologically not MEC; high grade in Brandwein, intermediate in Katabi and modified Healey, low in AFIP) who received adjuvant radiotherapy, recurred in the buccal region, and was alive with metastatic disease 22 years after surgery; patient 5 had a T4 NX palate carcinoma (morphologically MEC; high grade in Brandwein and modified Healey, intermediate in Katabi, low in AFIP) who was treated with surgery, had local recurrence in the neck 3 months after surgery, followed by

radiation therapy and additional neck and orbital recurrences; patient 6 had a T3 N2b submandibular carcinoma (morphologically MEC; high grade in all systems) who received adjuvant chemoradiation, recurred in the floor of mouth 2 months after resection, developed pulmonary metastases 4 months after resection, and died of disease 10 months after resection. Of the remaining 39 patients, follow-up ranged from less than 1 month to 13.3 years (average 3.82 years) and none recurred.

Follow-up of the 8 recurrent patients ranged from 0.8–26.9 years after recurrence (average 8.4 years). Three had additional loco-regional recurrences, one had distant metastasis, one had no additional recurrences, one had unexplained dysphagia and weight loss, one died at home of unknown causes, and one was lost to follow-up.

Risk Factors for Recurrence in Primary Carcinomas:

Morphology: Of the 40 carcinomas morphologically compatible with MEC, 4 (10%) recurred. Of the 5 morphologically non-MECs, 2 (40%) recurred. Morphology did not correlate to recurrence in primary carcinomas (p=0.1248) [Table 3I]. However, when considering all 53 carcinomas (including non-primary), those morphologically non-MEC were more likely to recur (7/10, 70%) compared to MEC (7/43, 16%) (p=0.0017) [Table 3J].

Margins: Of the 16 primary carcinomas with positive surgical margins, 3 (19%) recurred, all of which were Brandwein high grade, 2 of which were treated with adjuvant therapy. Of the remaining 13 with positive margins that did not recur, 5 (38%) were treated with adjuvant therapy, 7 (54%) with surgery only, and one was lost to follow-up. Of the 29 primary carcinomas with negative surgical margins, 3 (10%) recurred. One was treated with adjuvant radiation for close margins, one with adjuvant chemoradiation for N2b disease, and one with surgery only. Of the remaining 26 with negative margins that did not recur, one was treated with adjuvant radiation for skeletal muscle invasion (T3) and 25 were treated with surgery alone. Margin status did not correlate to recurrence (p=0.3585) [Table 3K]. Considering only primary carcinomas that were morphologically MEC (n=40), margin status still did not correlate to recurrence (p=0.1145) [Table 3L].

FISH: Of the 34 MAML2-rearranged cases, 3 (9%) recurred. Of the 5 non-rearranged cases, 0 (0%) recurred. FISH status did not correlate to recurrence (p=0.6548) [Table 3M]. Considering all carcinomas (including non-primary), FISH status also did not correlate to recurrence (p=0.0867) [Table 3N]. Of the 7 carcinomas in which hybridization failed, 4 (57%) recurred.

Stage: Of the 9 T4 carcinomas, 4 (44%) recurred. Of the 33 T1-T3 carcinomas, 1 (3%) recurred. T4 carcinomas were more likely to recur (p=0.0050) [Table 3O]. Considering only primary carcinomas that were morphologically MEC (n=38), T4 carcinomas were still more likely to recur (p=0.0237) [Table 3P].

Grade: Recurrence rates based on grade were highly dependent on the grading system used. Of the 13 primary carcinomas that scored high grade in the Brandwein system, 6 (46%) recurred; of the remaining 32 primary carcinomas that scored low-intermediate grade in all systems, none recurred (p=0.0002) [Table 3Q]. Considering only primary carcinomas that

were morphologically MEC (n=40), Brandwein high grade also correlated to recurrence (p=0.0014) [Table 3R]. Of the 11 patients with intermediate grade due to isolated solid growth, all received surgery only and none recurred after an average of 4.4 years (median 3.8, range 0.5–11.7 years).

Of the 45 primary cases graded in the Brandwein system, 0/32 (0%) low or intermediate grade and 6/13 (46%) high grade carcinomas recurred (p<0.0001) [Figure 4A, Supplementary Figure 3A]. Of these cases graded in the Katabi system, 0/22 (0%) low grade, 3/15 (20%) intermediate grade, and 3/8 (38%) high grade carcinomas recurred (p=0.0004) [Figure 4C]. Of these cases graded in the modified Healey system, 0/27 (0%) low grade, 3/10 (30%) intermediate grade, and 3/8 (38%) high grade carcinomas recurred (p=<0.0001) [Figure 4E]. Of these cases graded in the AFIP system, 3/36 (8%) low grade, 1/2 (50%) intermediate grade, and 2/7 (29%) high grade carcinomas recurred (p=0.0077) [Figure 4G, Supplementary Figure 3C].

Of the 40 primary cases that were morphologically MEC, the findings were similar: Of cases graded in the Brandwein system, 0/31 (0%) low or intermediate grade and 4/9 (44%) high grade carcinomas recurred (p<0.0001) [Figure 4B, Supplementary Figure 3B]. Of these cases graded in the Katabi system, 0/22 (0%) low grade, 1/12 (8%) intermediate grade, and 3/6 (50%) high grade carcinomas recurred (p<0.0001) [Figure 4D]. Of these cases graded in the modified Healey system, 0/26 (0%) low grade, 1/8 (13%) intermediate grade, and 3/6 (50%) high grade carcinomas recurred (p<0.0001) [Figure 4F]. Of these cases graded in the AFIP system, 1/33 (3%) low grade, 1/2 (50%) intermediate grade, and 2/5 (40%) high grade carcinomas recurred (p=0.00024) [Figure 4H, Supplementary Figure 3D].

Discussion:

In this evaluation of 53 historically-diagnosed mucoepidermoid carcinomas of the head & neck, a binary grading system (Brandwein high versus low-intermediate) best stratified patients into groups with differing risks of recurrence [Table 4]. Additionally, absence of *MAML2* rearrangement was not associated with recurrence, but non-mucoepidermoid morphology was.

These findings agree with prior studies reporting that the Brandwein system assigns higher grades to carcinomas and the AFIP system lower grades. The Katabi and modified Healey systems appear to grade tumors similarly and in an intermediate fashion between Brandwein and AFIP. Given the rate of recurrence of primary carcinomas that scored high grade in the Brandwein system (46% of all high grade carcinomas and 44% of morphologically mucoepidermoid high grade carcinomas), Brandwein high grade tumors could be considered biologically high grade, perhaps necessitating adjuvant therapy. Additionally, given the lack of recurrence in primary carcinomas that scored low-intermediate grade in the Brandwein system (0%), both Brandwein intermediate and low grade tumors could be considered biologically low grade, in which adjuvant therapy may not be necessary. This finding is consistent with other studies which have suggested that low and intermediate grade carcinomas behave similarly.[12,13,31] McHugh et al, using cystic versus solid growth and cellular anaplasia as main grading criteria in a cohort of 125 patients, found no differences in

disease-free or overall survival in low compared to intermediate grade cases.[13] Recurrence occurred in 11% of low grade, 9% of intermediate grade, and 57% of high grade cases. Nance et al, using the Brandwein system in 50 patients, also found no differences in disease-free or overall survival in low compared to intermediate grade.[12] Guzzo et al, graded 108 patients using the AFIP system, found no case that scored intermediate grade, and did find a significant difference in recurrence between low and high grade cases.[31]

In this study, 40% of primary carcinomas scored intermediate grade in at least one system, most frequently due to isolated solid growth (61%). None of these patients recurred after an average of 4.4 years. In light of these findings, a tumor that has only solid growth could be designated low grade. Conversely, perineural invasion, lymphovascular invasion, necrosis, increased mitoses, and anaplasia were only present in tumors scoring high grade in at least one system. A tumor that has any one of these features should be designated high grade. The features of peripheral invasion in nests and islands as well as bony invasion were present in both intermediate and high grade tumors, and may only helpful be in conjunction with other high grade features. While Katabi and modified Healey systems do stratify patients into three distinct grades with different recurrence rates, up to 30% of primary carcinomas scoring intermediate grade recurred. Management of these patients can therefore be uncertain. Of the 6 patients scoring high in Brandwein but intermediate in Katabi or modified Healey, 4 recurred. Brandwein grade (high versus low-or-intermediate) was a better independent predictor of recurrence. Finally, the AFIP system was the least accurate at predicting recurrence and should be avoided. For these reasons, a binary grading system for mucoepidermoid carcinoma should be considered using the Brandwein system: histologically high grade should be considered biologically aggressive and histologically low-or-intermediate grade should be considered biologically indolent or low grade. Only two other studies have compared histologic grading systems in mucoepidermoid carcinomas. Qannam et al compared 19 MECs across all 4 systems and found overall low (32%) agreement, similar to that in our study (43%). They found that the AFIP and Katabi systems yield lower grades while Brandwein and modified Healey assign higher grades.[32] Their findings agree with ours, however, they did not correlate grade to recurrence rates. Bai et al compared 79 MECs using AFIP and Brandwein systems and found overall 41% of cases were upgraded from AFIP to Brandwein.[33] They also did not compare the predictive performance of the grading systems.

Additional clinicopathologic features predictive of recurrence were evaluated in our study. In primary carcinomas, pathologic stage T4 was associated with recurrence but margin status was not. Stage T4 was not associated with MEC morphology or *MAML2* rearrangement. Evaluating all carcinomas, presence of *MAML2* rearrangement was not associated with recurrence, but morphology was: carcinomas morphologically compatible with MEC were less likely to recur. These findings are likely explained by a few confounding factors: tumors that are low grade are more likely to be accurately classified by morphology as true MEC (97% Brandwein low-intermediate grade compared to 55% high grade were morphologically MEC and 93% Brandwein low-intermediate grade compared to 56% high grade were *MAML2* rearranged). Tumors that are high grade may be given a diagnosis of MEC in the absence of ideal histologic features or *MAML2* gene rearrangement, simply for lack of a more specific diagnosis [Figure 3]. Morphologic features compatible with MEC (regardless

of grade) included abundant intermediate cells, multifocal presence of cells with true intracytoplasmic mucin, and usually combined solid and cystic growth (either macro or microcysts). Squamoid cells were the least in abundance. The application of a mucicarmine or PAS/PAS-diastase stain could facilitate in the identification of true intracytoplasmic mucin rather than clearing due to glycogen. Additionally, carcinomas with architectural high grade features (perineural invasion lymphovascular invasion, necrosis) were occasionally cytologically bland. Morphologic features compatible with MEC were strongly associated with presence of *MAML2* gene rearrangement, such that all 37 *MAML2*-rearranged carcinomas were morphologically MEC. Only 3 cases that were morphologically MEC were not rearranged, and all had intermediate cells and intracellular mucin [Figure 2]. In these cases, absence of *MAML2* rearrangement could be explained by inherent limitations of FISH to detect a rearrangement or absence of *MAML2* rearrangement in a small subset of mucoepidermoid carcinomas. Recent whole-exome sequencing has demonstrated *TP53* mutations in some *MAML2* wildtype MECs.[34]

The prevalence and prognostic significance of *CRTC1-MAML2* or *CRTC3-MAML2* rearrangement in MEC has been studied with varying results. Prevalence rates ranging from 34% to 82% have been reported, with *CRTC1* being the more common partner.[14–22,24–29,35] Better prognosis in *MAML2*-rearranged carcinomas has been shown, mostly in studies with low rearrangement rates.[14–16,18–22] Five of these eight studies had *MAML2* rearrangement rates 55%. Other studies showing similar prognosis between *MAML2*-rearranged and non-rearranged carcinomas had rates between 56% and 75%.[24,25,28,29] In this study, *MAML2* rearrangement was present in 93% of cases morphologically compatible with MEC, and rearrangement did not correlate with recurrence. It has been suggested that non-rearranged tumors may represent non-mucoepidermoid carcinomas, some of which may be *EWSR*-rearranged clear cell carcinomas. High grade carcinomas of uncertain morphology may have a higher tendency to be called MEC even in the absence of the combined features of intracellular mucin, intermediate cells, and squamoid cells.[16,24,25,28,30,36] Therefore, when restricting the diagnosis of MEC to those with certain morphology, the prognostic significance of *MAML2* rearrangement diminishes. Mucoepidermoid carcinomas with *MAML2*-rearrangement may also behave aggressively, and some have suggested that *MAML2*-rearrangement in the presence of a complex genome[26], *HMGA2* expression[17], or *CDKN2A* loss[23] portends worse prognosis. Further characterization of the grading systems in the presence of genomic information is warranted.

Histologic grading across all four MEC grading systems was inconsistent. However, Brandwein high grade is likely the best predictor of aggressive behavior. A binary grading system is proposed: cases scoring low or intermediate grade in the Brandwein system could be considered biologically low grade; cases scoring Brandwein high grade as biologically high grade. The most likely histologic finding for an increase to intermediate grade was solid growth, but behavior in these patients compared to those with low grade disease did not change. In the setting of isolated solid growth, upgrading to intermediate may not be warranted. Lastly, presence of *MAML2* rearrangement was highly correlated to MEC morphology but not to recurrence. High grade non-rearranged carcinomas are likely morphologic mimics of MEC, and prior studies demonstrating worse prognosis in *MAML2*-

non-rearranged MECs were likely diluted by high grade non-MECs. Strict histologic criteria should be used when rendering a diagnosis of MEC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

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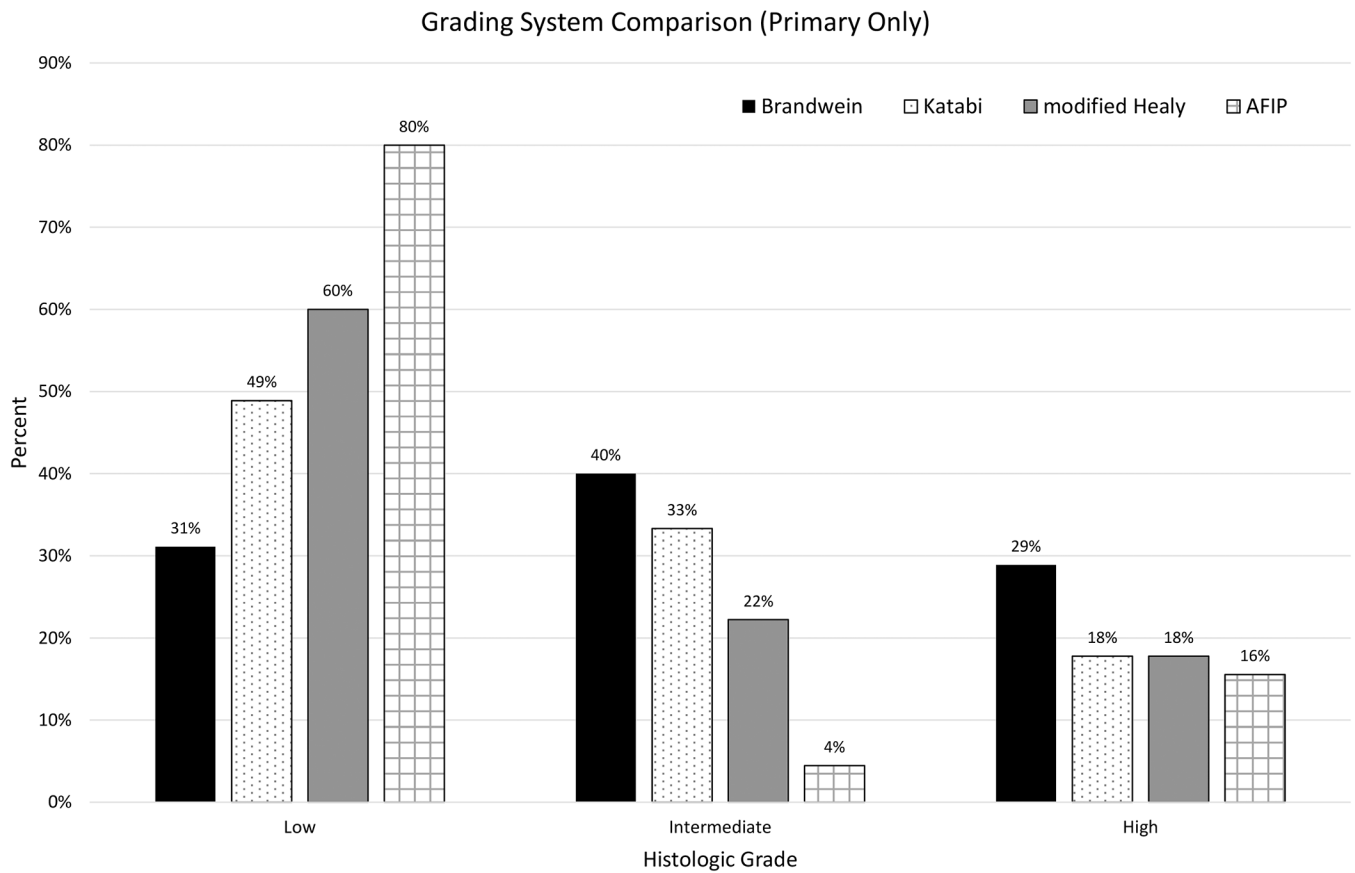
Figure 1: Four Mucoepidermoid Carcinoma (MEC) Grading Systems Compared
Forty-five primary carcinomas (of which 40 were morphologically compatible with MEC on contemporary re-review) were graded according to four published grading systems. Brandwein assigned the highest rate of high grade (29%), while AFIP the highest rate of low grade (80%). The distribution was similar after eliminating the 5 non-MEC cases [Supplementary Figure 2].
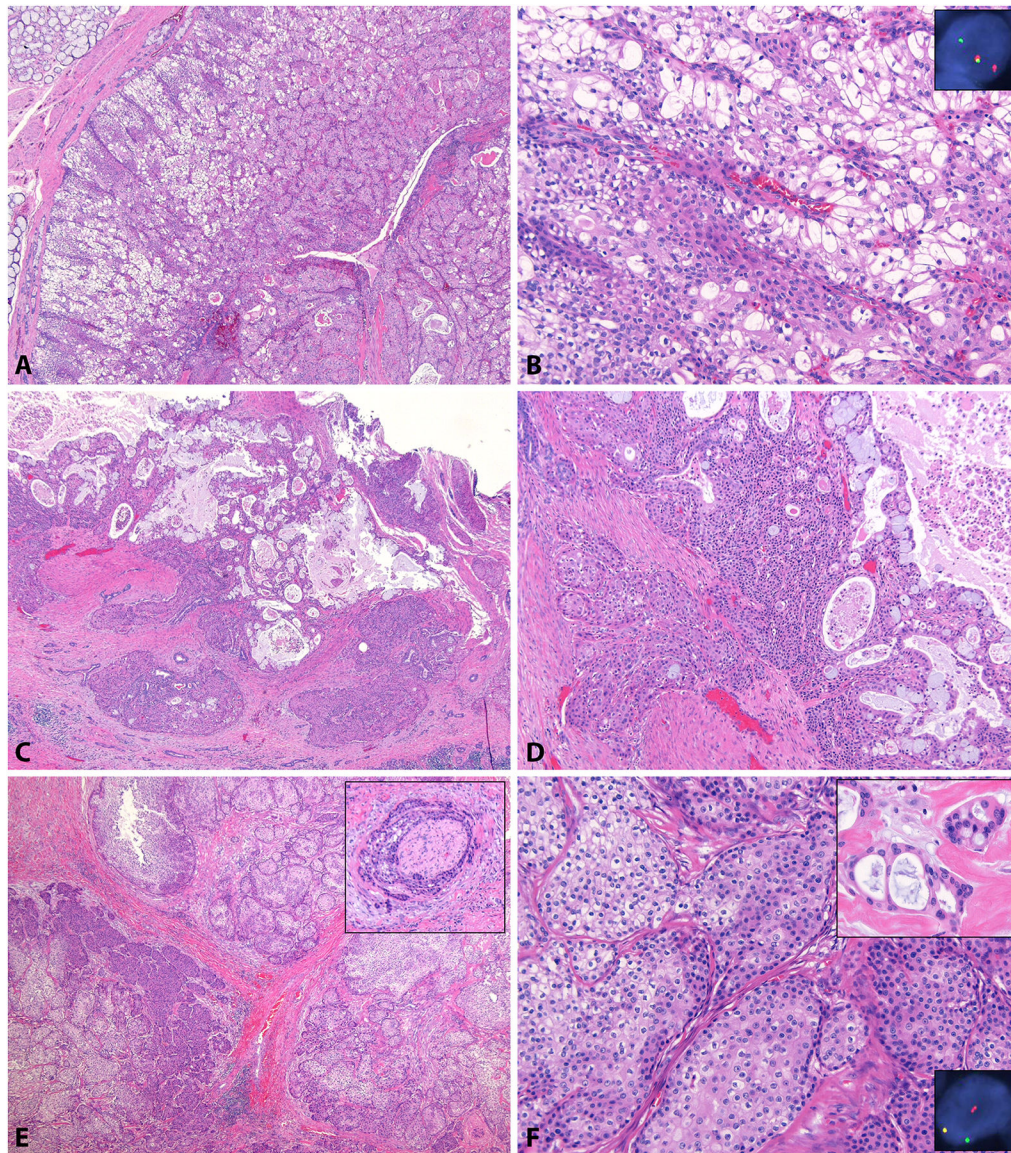
Figure 2: Carcinomas with Mucoepidermoid Morphology

Most (81%) carcinomas reviewed were morphologically compatible with MEC. Solid growth was the most common reason for assigning Brandwein intermediate grade, as seen in this well-circumscribed (A) mucoepidermoid carcinoma with intermediate cells, glycogenated cells (B), rare mucinous cells, and *MAML2* rearrangement (B, inset). Occasional carcinomas morphologically consistent with MEC (C), showed intermediate cells, squamoid cells, and mucinous cells (D) but lacked *MAML2* rearrangement. A true high grade mucoepidermoid carcinoma also showed appropriate cell types and high grade features, including perineural invasion (E, inset), rare mucinous cells (F, top inset), and *MAML2* rearrangement (F, bottom inset).
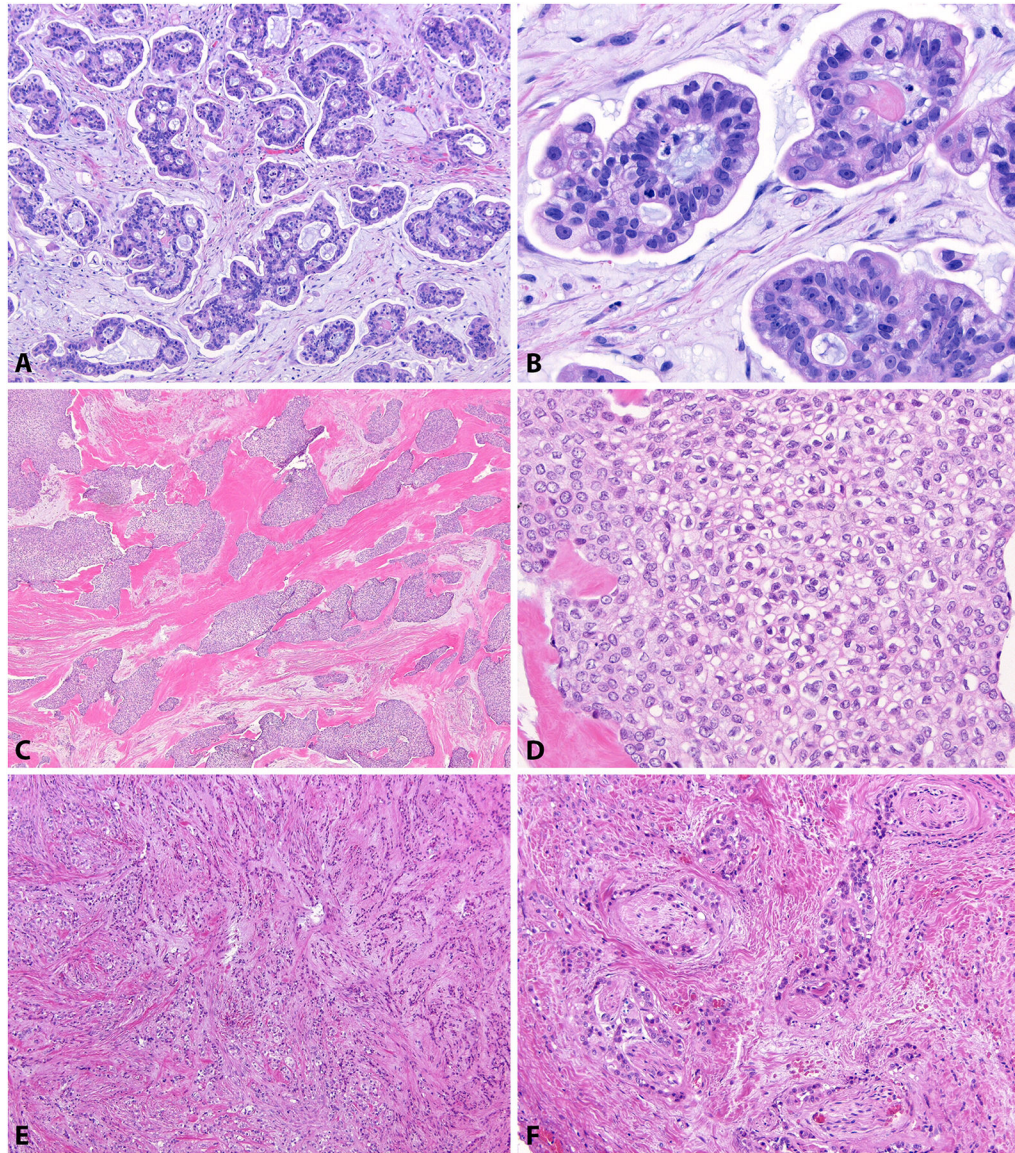
Figure 3: Carcinomas of Non-Mucoepidermoid Morphology

Some carcinomas (19%) originally diagnosed as MEC by the signout pathologist were not compatible with MEC upon contemporary review. This primary palate carcinoma showed micropapillary architecture (A) with extracellular mucin pools (B) and was negative for *MAML2* rearrangement, suggestive of the rare mucinous adenocarcinoma. A buccal carcinoma demonstrated abundant eosinophilic hyalinized stroma (C) and a single cell type with clear cytoplasm and wrinkled nuclei (D), consistent with clear cell carcinoma. Another palate carcinoma also showed a single cell type with single-file infiltration within fibrous stroma (E) and perineural invasion (F). The remaining non-MEC cases were not morphologically specific for other salivary type carcinomas.
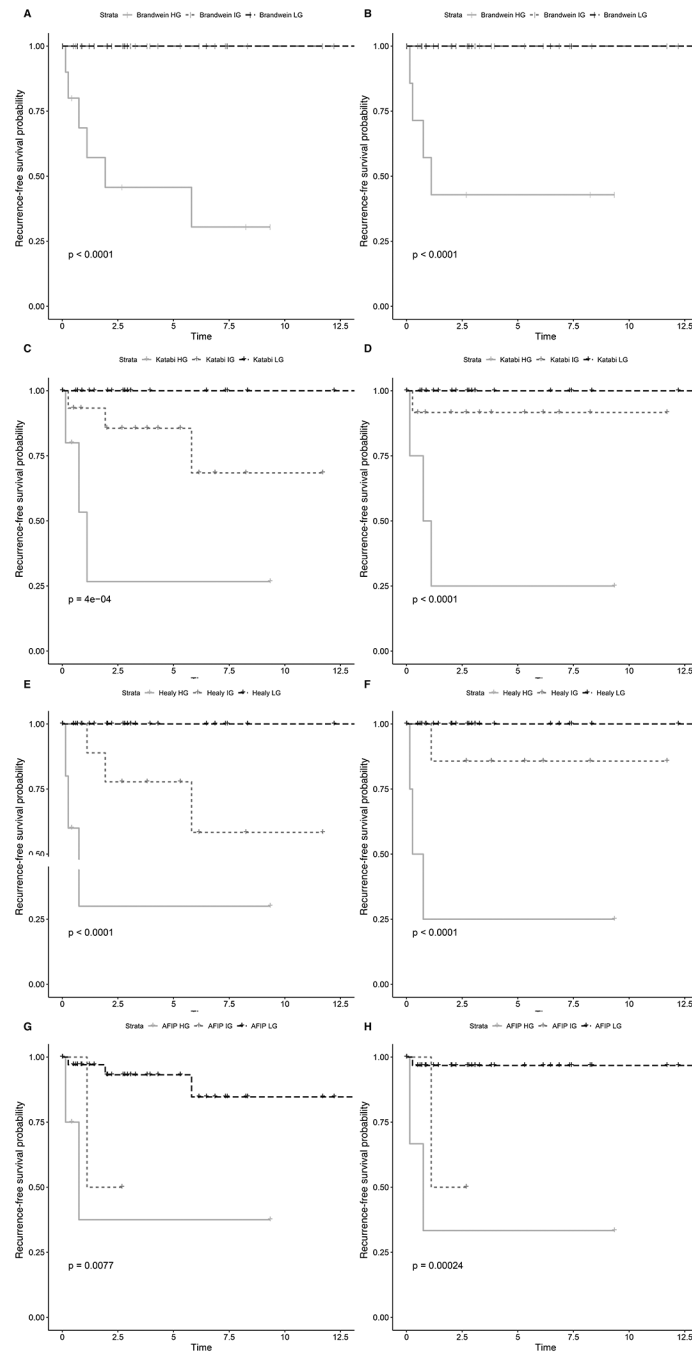
Figure 4: Kaplan-Meier Recurrence Curves Based on Ternary Grading of Primary Cases
Considering all 45 primary cases, Brandwein intermediate grade carcinomas behaved similarly to low grade, with no recurrences in either group. Recurrences only occurred in the high Brandwein grade group (A). A binary Brandwein system could be employed as an improved predictor of recurrence [Supplementary Figure 3A]. In the Katabi (C) and modified Healy (E) systems, low, intermediate, and high grade carcinomas had different recurrence-free survival rates, including no recurrences in the low grade groups. AFIP low and intermediate grade carcinomas had worse recurrence-free survival rates compared to

other systems, suggesting that this system undergrades carcinomas (G). Additionally, AFIP intermediate grade carcinomas behaved similarly to high grade [Supplementary Figure 3C]. Considering only the 40 primary carcinomas morphologically compatible with mucoepidermoid, the findings are very similar (B, D, F, H) [Supplementary Figure 3B, 3D].

Table 1:

Comparison of Mucoepidermoid Carcinoma Histologic Grading Systems

	Modified Healey[5,6]	AFIP[7,8]	Brandwein[9]	Katabi[10]
Intracystic Component	L: macro + micro cysts I: micro cysts + solid H: solid +/− micro cysts	2 (<20%)	2 (<25%)	L: predominantly cystic (>80%) I: predominantly solid H: any (usually solid)
Perineural Invasion	H: present	2	3	n/a
Necrosis	n/a	3	3	L: absent I: absent H: present
Mitosis	L: rare I: few H: many	3 (4/10 HPF)	3 (5/10 HPF)	L: 0–1/10 HPF I: 2–3/10 HPF H: 4+/10 HPF
Nuclear Anaplasia / Pleomorphism	L: absent/minimal I: slight/moderate H: considerable (including nucleoli)	4	2	L: no significant I: no significant H: any
Border / Invasive Front	L: broad/circumscribed I: uncircumscribed H: soft tissue/perineural/vascular invasion	n/a	2 (small nests & islands)	L: well circumscribed I: well circumscribed or infiltrative H: any (usually infiltrative)
Lymphovascular Invasion	H: present	n/a	3	n/a
Bony Invasion	n/a	n/a	3	n/a
Intermediate Cells	L: rare I: more common H: predominant	n/a	n/a	n/a
Stroma	L: extravasated mucin + fibrosis + CI I: fibrosis separating nests + CI H: desmoplasia, minimal CI	n/a	n/a	n/a
Architecture	L: daughter cysts from larger I: large duct less conspicuous H: variable architecture/cell morphology	n/a	n/a	n/a
Low		0–4	0	
Intermediate		5–6	2–3	
High		7–14	4+	

Key: L=low grade, I=intermediate grade, H=high grade, n/a=not applicable, CI=chronic inflammation.

Table 2:

Clinicopathologic Features of Mucoepidermoid Carcinoma Patients

	Morphologically-MEC (n=43)	Morphologically-Not-MEC (n=10)
Specimen Type		
- Excision of primary or initial re-excision	40	5
- Excision of recurrence	3	5
Clinical Features		
Site, all		
- Minor salivary gland	26	6
- Major salivary gland	17	4
Average Age (years)		
- Primary	52	58
- Recurrent	31	61
Sex		
- Female	29	5
- Male	14	5
Treatment, primary cases		
- Surgery only	32	3
- Surgery + RT	3	2
- Surgery + CRT	5	0
Recurrence, primary cases	4	2
Average Follow-up (years), primary cases, non-recurrent	4.0	1.6
Pathologic Features		
Average Size (cm), primary cases	1.8	1.6
Pathologic Stage, primary cases	30 pT1–3, 8 pT4, 2 pTX	3 pT1, 1 pT4, 1 pTX
Margins Involved, primary cases	14	2
Original Grade, primary cases	3 HG, 10 IG, 27 LG	2 HG, 2 IG, 1 LG
Reviewed Brandwein Grade, primary cases	9 HG, 17 IG, 14 LG	4 HG, 1 IG
Reviewed Brandwein Grade, recurrence cases	2 HG, 1 LG	5 HG
MAML2 Status		

	Morphologically-MEC (n=43)	Morphologically-Not-MEC (n=10)
- Rearranged	37	0
- Not-Rearranged	3	6

Key: MEC=mucoepidermoid, RT=radiotherapy, CRT=chemoradiotherapy, F/U=follow-up, HG=high grade, IG=intermediate grade, LG=low grade.

Statistical Analyses of Mucoepidermoid Carcinoma Patients

Table 3:

A

n=53 all	Brandwein LG-IG	Brandwein HG		Fisher's exact	p=0.0003
Morphologically non-MEC	1	9	90.0%		
Morphologically MEC	32	11	25.6%		
	97.0%	55.0%			

B

n=45 primary	Brandwein LG-IG	Brandwein HG		Fisher's exact	p=0.0198
Morphologically non-MEC	1	4	80.0%		
Morphologically MEC	31	9	22.5%		
	96.9%	69.2%			

C

n=42 primary	T1-T3	T4		Fisher's exact	p=0.6344
Morphologically non-MEC	3	1	25.0%		
Morphologically MEC	30	8	21.1%		
	90.9%	88.9%			

D

n=46 all	MAML2-WT	MAML2-rearranged		Fisher's exact	p=0.000008
Morphologically non-MEC	6	0	0.0%		
Morphologically MEC	3	37	92.5%		
	33.3%	100.0%			

E

n=46 all	MAML2-WT	MAML2-rearranged		Fisher's exact	p=0.0049
Brandwein LG-IG	2	28	93.3%		
Brandwein HG	7	9	56.3%		
	77.8%	24.3%			

F

n=37 primary	MAML2-WT	MAML2-rearranged		Fisher's exact	p=0.5297
Brandwein LG-IG	2	27	93.1%		
Brandwein HG	1	7	87.5%		
	33.3%	20.6%			

G

n=37 primary	MAML2-WT	MAML2-rearranged		Fisher's exact	p=0.6102
T1-3	4	27	87.1%		
T4	1	5	83.3%		
	20.0%	15.6%			

H

n=35 primary morph MEC	MAML2-WT	MAML2-rearranged		Fisher's exact	p=0.4417
T1-3	2	27	93.1%		
T4	1	5	83.3%		
	33.3%	15.6%			

I

n=45 primary	Non-Recurrent	Recurrent		Fisher's exact	p=0.1248
Morphologically non-MEC	3	2	40.0%		
Morphologically MEC	36	4	10.0%		
	92.3%	66.7%			

J

n=53 all	Non-Recurrent	Recurrent		Fisher's exact	p=0.0017
Morphologically non-MEC	3	7	70.0%		
Morphologically MEC	36	7	16.3%		
	92.3%	50.0%			

K

n=45 primary	Non-Recurrent	Recurrent		Fisher's exact	p=0.3585
Margins Uninvolved	26	3	10.3%		
Margins Involved	13	3	18.8%		
	33.3%	50.0%			

L

n=40 primary morph MEC	Non-Recurrent	Recurrent		Fisher's exact	p=0.1145
Margins Uninvolved	25	1	3.8%		
Margins Involved	11	3	21.4%		
	30.6%	75.0%			

M

n=39 primary	Non-Recurrent	Recurrent		Fisher's exact	p=0.6548
MAML2-WT	5	0	0.0%		
MAML2-rearranged	31	3	8.8%		
	86.1%	100.0%			

N

n=46 all	Non-Recurrent	Recurrent		Fisher's exact	p=0.0867
MAML2-WT	5	4	44.4%		
MAML2-rearranged	31	6	16.2%		
	86.1%	60.0%			

O

n=42 primary	Non-Recurrent	Recurrent		Fisher's exact	p=0.0050
T1-T3	32	1	3.0%		
T4	5	4	44.4%		
	13.5%	80.0%			

P

n=38 primary morph MEC	Non-Recurrent	Recurrent		Fisher's exact	p=0.0237
T1-T3	29	1	3.3%		
T4	5	3	37.5%		
	14.7%	75.0%			

Q

n=45 primary	Non-Recurrent	Recurrent

R

n=40 primary morph MEC	Non-Recurrent	Recurrent

Brandwein LG-IG	32	0	0.0%	Fisher's exact
Brandwein HG	7	6	46.2%	p=0.0002
	17.9%	100.0%		

Brandwein LG-IG	31	0	0.0%	Fisher's exact
Brandwein HG	5	4	44.4%	p=0.0014
	13.9%	100.0%		

Key: LG=low grade, IG=intermediate grade, HG=high grade, MEC=mucoepidermoid carcinoma, WT=wildtype.

Table 4:

Clinicopathologic Features of Primary Morphologically Mucoepidermoid Carcinomas Using Proposed Binary Grading System

Parameter (n=40)	Brandwein Low-Intermediate Grade (n=31)	Brandwein High Grade (n=9)	p-value
Site (Minor Salivary Gland)	21 (67.7%)	3 (33.3%)	0.1198
Average Age, Years	50.8 (range 9–75)	56.7 (28–85)	0.3782
Sex (Female)	21 (67.7%)	6 (66.7%)	1
Average Tumor Size, cm	1.4 (0.3–3.8)	3.4 (1.2–6.8)	<0.0001
Margins Positive	9 (29.0%)	5 (55.6%)	0.2338
Stage (pT4)	4 (12.9%)	4 (44.4%)	0.0594
Treatment (Surgery Alone)	28 (90.3%)	3 (33.3%)	0.0014
Recurrence	0	4 (44.4%)	0.0014
MAML2-rearranged	27/29 (93.1%)	7/8 (87.5%)	1