Immunohistochemical differentiation of Adenoidcystic Carcinoma from Polymorphous Low-Grade Adenocarcinoma using Ki67 and Alpha-SMA

Introduction

Adenoidcystic Carcinoma (ADCC) and Polymorphous Low-grade Adenocarcinoma (PLGA) are two malignant salivary gland tumours that show many histopathologic and some clinicopathologic features in many respect. However, they are dissimilar in clinical behavior. The PLGA is a relatively new entity [1,2].

The ADCC is a highly malignant neoplasm of the salivary gland with little cytological atypia except in the solid pattern. There are two cell types implicated in this malignancy; the ductal epithelial cells and the myoepithelial cells. Different pattern of histological appearance are seen (ductal, cribriform, solid and tubular). It is prone to widespread recurrences and metastasis. It has very poor prognosis even after combination therapy [1,2]. It occurs in both major and minor salivary glands.

The PLGA on the other hand has a low propensity for metastasis but can be infiltrative. It also has varying histologic patterns (cribriform, papillary, papillary cystic, lobular and trabecular patterns) as described by Norberg et al [3]. It has a uniform cytologic appearance. Recurrence is common particularly the palatal lesion. Both ADCC and PLGA are difficult to differentiate in minor salivary gland especially the palatal minor salivary gland [2-5]. This occurs mainly in the minor salivary glands and very rarely in major salivary glands.

Both have propensity to infiltrate the nerves and therefore have pain as early symptoms [1,3]. The overlapping histological features of ADCC and PLGA occasionally may result in a diagnostic pitfall and especially when small biopsies do not contribute to distinguish between these tumors.

Various methods have been used to differentiate PLGA from ADCC. Such methods include immunohistochemical method using monoclonal antibodies using α-SMA, Ki67, p63, c-kit and many others [5-15].

C-Kit is a stem cell growth factor (SCFR) or a proto-oncogene c-ktor a receptor tyrosine kinase protein found in humans and encoded in kit gene and is a new
monoclonal antibody which is currently used in distinguishing ADCC from PLGA because ADCC shows a lot of affinity to it whereas PLGA is negative to c-Kit [14,15].

α-SMA and Ki67 have been used by many researchers to distinguish ADCC from PLGA. Ki67 is said to be positive to both ADCC and PLGA but at varying degrees. Ki67 showed more intense positive reaction to ADCC than to PLGA as reported by Vargas et al [11]. It was also observed that α-SMA was positive in ADCC. The reason for this positivity was the myoepithelial cells that play a role in its histogenesis [5-9].

Since the results of many markers currently being used to differentiate these lesions is not consistent, we therefore designed this study to determine the role of α-SMA and Ki67 in the diagnosis of ADCC and PLGA.

### Materials and Methods

Seven histopathologically diagnosed ADCC and four histopathologically diagnosed PLGA were retrieved and processed for immunohistochemistry. All specimens were previously fixed in 10% neutral formalin and embedded in paraffin wax. Anti α-SMA and anti Ki67 were the immunostaining agents used.

Avidin Biotin Complex (ABC) method were used. All antibodies used were manufactured by Novocastra (Novocastra product now owned by LEICA). ELIZA method was used and manufacturer instruction was strictly followed.

The antibody dilution was 1:100 for all antibody markers.

Tissues for processing were sectioned at 2µm on the rotatory microtome and placed on the hot plate at 70 degree for one hour. Sections were passed in xylene and graded alcohol. Antigen retrieval was done by heating on a citric acid solution at PH 6.0 in a microwave for 15 minutes. Sections were equilibrated gradually with cool water to displace hot citric acid for 5 minutes for the sections to cool. Sections were covered with 3% hydrogen peroxide for 15 minutes to block the peroxidase activity. Sections were washed with PBS and protein blocking were performed using avidin for 15 minutes. Sections were washed in PBS and endogenous biotins in tissue were blocked using biotin for 15 minutes.

Excess antibody were washed off with PBS and secondary antibody (LINK) applied for 15 minutes. Sections were washed and the LABEL which is the horseradish peroxidase (HRP) was applied on the sections for 15 minutes.

The working DAB solution was made up of 1 drop (20µ) of the DAB chromogen to 1ml of DAB substrate. This solution was applied on sections after washing off the HRP with PBS for 5 minutes. Excess DAB solution and precipitate were washed off with water.

Sections were counterstained with haematoxylin solution for about 2 minutes and blued briefly. Sections were dehydrated in alcohol, cleared in xylene and mounted.

For each reaction, it was reported either positive (when there was reaction) or negative (no reaction). When positive, it was graded mild (+1), moderate (+2) or intense (+3) depending on the intensity and extent of the positive reaction. Statistical analysis was done with SPSS version 20; descriptive statistics (frequency, tables) were done. Association between variables were accessed with Chi-square and P-value was set at <0.05.

### Results

The seven PLGA were all (100%) negative to alpha SMA (Figure 1) while the ADCC were all positive to alpha SMA (Figure 2). One ADCC (25%) was moderately positive to α-SMA while 3 (75%) were intensely positive to α-SMA. The seven PLGA were all negative (100%) to α-SMA. All positivities were both nuclear and cytoplasmic. The difference in sensitivity between ADCC and PLGA to α-SMA was statistically significant (p-value=0.004).

Five PLGA (71.4%) were moderately positive to Ki67 while two (28.6%) were intensely positive to Ki67 (Figure 3). Three monoclonal antibody which is currently used in distinguishing ADCC from PLGA because ADCC shows a lot of affinity to it whereas PLGA is negative to c-Kit [14,15].

### Materials and Methods

#### Avidin Biotin Complex (ABC) method

- Sections were treated with 3% hydrogen peroxide for 15 minutes to block the peroxidase activity.
- Sections were washed with PBS and protein blocking was performed using avidin for 15 minutes.
- Sections were washed in PBS and endogenous biotins in tissue were blocked using biotin for 15 minutes.

### Table 1: Showing the Reactions of Adcc and Plga to Antigenic Markers (α-Sma And Ki67).

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<th>TYPE OF TUMOUR</th>
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<th>PLGA</th>
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<table>
<thead>
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<th>TYPE OF TUMOUR</th>
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agreement with the findings of Saghravanian, et al. [5]. This may appear to be a tool to differentiate between the two lesions. However, Beltran, et al. [7] concluded in their study that c-kit is better in distinguishing between ADCC and PLGA.

The positivity of ADCC to α-SMA according to Prasad et al. [9] is due to immunoreactivity to myoepithelial cells in the lesion [16].

Three (75%) ADCC were strongly positive to Ki67 while one (25%) was moderately positive to it. Five (71.4%) PLGA were moderately positive to Ki67 and two (28.6%) were strongly positive to Ki67. The Ki67 is a proliferative marker for proliferating cells and a useful marker for malignant diseases. It’s over expression indicate poor prognosis. The difference in the sensitivity between ADCC and PLGA to Ki67 was not statistically significant (p =0.137). This is not in agreement with the finding of Saghravanian, et al. [5] who found significance difference in the reactivity of ADCC and PLGA to Ki67. This is also in contradiction to the findings of Gnepp, et al. [6] and Skalova, et al. [8] who found that Ki67 was useful in distinguishing ADCC from PLGA. Darling, et al. [17] reported indistinct and lower expression of Ki67 in PLGA in their study and therefore concluded that Ki67 was a useful tool in differentiating ADCC from PLGA since the ADCC was very positive to Ki67. This was also corroborated by Beltran, et al. [10].

The positivity of ADCC to α-SMA confirms the role of myoepithelial cells in the histogenesis of ADCC while the negative reactions of PLGA suggests that myoepithelial cells do not have any role in the histogenesis of PLGA [9,10] although some authors have shown the positivity of PLGA to α-SMA [16,17]. It was actually negative to all smooth muscle antibody markers (SMMH and Calponin) used in the study of Prasad, et al. and Prasad, et al. [9,10].

Discussion

The ADCC and the PLGA have many similarities in their histopathologic characteristics but differ to a great extent in their clinical behaviours. ADCC is composed of ducal epithelial cells and myoepithelial cells which have angular and hyperchromatic nuclei and clear cytoplasm. Its histologic picture seems innocuous without nuclear atypia and abnormal mitosis but very invasive with numerous local recurrences and metastases and poor prognosis even after combination therapy [1,2,7-9].

The PLGA on the other hand is made up of malignant epithelial cells of cellular uniformity but diverse morphological presentation and infiltrative outlook. There are various histological presentations consisting of lobular, papillary, papillary cystic, trabeculae and cribriform patterns [1-3]. Like the ADCC it invades both the nerves and the vascular channels leading to experience of pain in both of them. Both of them (ADCC and PLGA) are particularly common in the minor salivary glands and in particular the palate where there is always difficulty in distinguishing between the two [1,2,7-14].

In this study, three (75%) ADCC were intensely positive to α-SMA while one (25%) was moderately positive to α-SMA. The seven PLGA (100%) were all negative to α-SMA. This is in agreement with the findings of Saghravanian, et al. [5]. This may appear to be a tool to differentiate between the two lesions. However, Beltran, et al. [7] concluded in their study that c-kit is better in distinguishing between ADCC and PLGA.

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Conclusion

α-SMA appears to be a useful tool in differentiating ADCC from PLGA when the histological finding is unequivocal. In our study, Ki67 appeared to have no role in distinguishing these two lesions, however bigger population is needed to confirm this finding.

References


