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Original Article

Pattern of invasion in squamous cell carcinomas of the lower lip and oral cavity

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ABSTRACT

Background: A number of factors may be responsible for the differences in the biologic behaviors of oral and lower lip squamous cell carcinomas (SCCs). Immunohistochemical invasion profiles have been used to detect invasion patterns like epithelial-mesenchymal-transition (EMT) and collective-cell-invasion (CCI), which have not been investigated in lower lip neoplasms. The aim of the present study was to compare the invasive phenotypes of SCCs of the lower lip and oral cavity.
Method: A total of 44 OSCCs and 37 lower lip SCCs were immunostained with E-Cadherin, N-Cadherin, and podoplanin. Based on their expression patterns, tumors were allocated to EMT, CCI or non-EMT/non-CCI categories.
Results: None of the oral SCCs showed EMT; while 5 lower lip SCCs demonstrated this phenotype. CCI was observed in 12 oral SCCs and 4 lower lip SCCs. The third group included 32 and 28 cases of oral and lower lip tumors, respectively. A significant difference in invasive phenotype was found between the two locations ($P = 0.009$).
Conclusion: Oral cavity and lip tumors differ in various aspects and according to our results; the pattern of invasion may be added to these features. Between the two major invasion patterns, EMT was more prevalent in lip tumors while CCI was observed more commonly in oral neoplasms. The significance of the different expression patterns of the non-EMT/non-CCI category requires further investigation.

1. Introduction

The ability of neoplastic cells to infiltrate, invade and penetrate adjacent tissues can ultimately result in distant and local metastases, which is a distinctive feature of cancers and a determinant of prognosis in malignancies.[1,2] Evaluation of recognized immunohistochemical invasion profiles has been suggested for determining the infiltrative pattern of tumors.[1,3]

Studies have shown that various invasion patterns differ in the cellular/molecular events that take place between migration initiation and the final penetration of neoplastic cells into normal surrounding tissues. When solitary cells invade the underlying stroma, they undergo epithelial-mesenchymal transition (EMT) which disrupts the expression and function of adhesion proteins so that tumor cells lose their epithelial molecules like E-Cadherins (ECads) and acquire mesenchymal markers such as N-Cadherins (NCads), also known as the cadherin switch.[4,5] This replacement results in fundamental changes in cellular behavior: the cell loses its ability to adhere to neighboring epithelial cells and by acquiring NCad, its capacity for migration and invasion, increases.[6] It is noteworthy that these exact changes may not always be discernible in the invasive margin and despite the absence of ECad in tumor cells, NCad might not be expressed, leading to the suggestion of incomplete EMT in such cases.[2] Yet another scenario could be the preservation of cell-to-cell contacts without the development of mesenchymal markers, which puts forward the possibility of collective cell invasion (CCI).[2,3] Podoplanin (Pod) has been suggested to play an essential role in CCI and its expression in the invasive front of tumors, may be associated with this type of invasion.[4]

Cadherins have been previously investigated in oral cavity squamous cell carcinomas (OSCCs), reporting expression rates of 37–92% for NCad and a decreased expression of ECad in the invasive margin, leading to the speculation of EMT in these tumors.[7,8] On the contrary, a number of studies have detected limited expression of NCad in oral and oropharyngeal SCCs with 90% negativity for this marker. These investigations suggested that the probability of complete EMT in OSCC is very low.[9,10] In line with these reports, Pod was used alongside E- and N-Cads to evaluate invasive patterns of OSCC and the results indicated the probability

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of CCI in these cancers.[3,11] A plethora of molecular pathways and markers is involved in the invasion of cancer cells, however the most popular proteins to define EMT and CCI have been the cadherin family, mesenchymal markers like vimentin and the trans-membrane glycoprotein Pod due to its possible role in CCI.[3,4,5,8,12]

There are limited studies on the invasion pattern of OSCC[3,12]; however, similar investigations in lip SCCs have not been performed. OSCC typically has a worse prognosis than lower lip SCC[13,14] which might be related to a number of factors including their different molecular expression profiles. Neoplastic invasion that is known to occur in two main patterns of EMT and CCI with specific immunohistochemical profiles[3,4,12] is an important factor in metastasis which has a major effect on prognosis. Due to the differences in the biologic and clinical behaviors of oral and lip SCCs, various neoplastic aspects have been compared between these sites.[13,14] Conforming to these studies we aimed to compare the invasion modes of lower lip and OSCCs, using the immunohistochemical expression of ECad, NCad, and Pod proteins. We were not able to find previous research in this regard.

2. Materials and methods

2.1. Samples

This retrospective study was performed on tissue blocks from primary, completely excised oral and lower lip SCCs retrieved from the pathology archive of our Institution from 2011 to 2015. None of the patients involved with cancer, had concomitant tumors or metastasis elsewhere and they had not received chemotherapy, radiotherapy, or any other treatment prior to surgery. Samples with significant necrosis and inadequate tissue were excluded from the investigation. The Ethics Committee of our University approved the protocol for this research.

2.2. Immunohistochemical staining procedures

Staining was performed as explained previously.[3] In brief, positively charged slides were used to mount 4 μm sections and were subsequently subjected to xylene deparaffinization and rehydration. All specimens were washed in tap water, submerged in phosphate buffered saline (PBS), immersed in 3% H_2O_2 for hydrogen peroxide blocking and rinsed in PBS. Following treatment with 10 mM citrate buffer (pH = 6), antigen retrieval was performed in a microwave oven for 5 and 15 min at high and low power, respectively. After 20 min cooling at room temperature, the slides were washed with PBS and incubated with primary monoclonal mouse antihuman antibodies against Podoplanin (D2-40; Dako, Glostrup, Denmark; 1:100 dilution), NCad (6G11; Dako, Glostrup, Denmark; 1:50 dilution) and ECad (NCH-38; Dako, Glostrup, Denmark; Ready-to-Use). The EnVision System (Dako Cytomation, Glostrup, Denmark) was used for detection of bound antibody by incubation for 30 min at room temperature. Positive controls consisted of a tissue known to contain abundant lymph vessels and internal lymph vessels for Pod, normal oral mucosa for ECad and stomach tissue for NCad. These were run simultaneously with the negative controls (omission of primary antibodies) and sample slides.

2.3. Analysis of immunostained sections

According to our previous study,[3] both cadherins were scored at the invasive front by assessment of staining intensity and proportion of positive tumor cells. For the former, lack of immuostaining was assigned a score of 0, while weak, moderate and strong positivity were given scores of 1–3, respectively. The latter was classified into 0 where <1% of tumor cells stained positive; 1, if ≥1–<40% were positive; 2, when ≥40–<80% showed immunoreactivity and 3 in cases with ≥80% staining of neoplastic cells. The multiplication of these values was rated as negative (0), weak (1–2), moderate (3–4) and strong (6–9). Negative and weak tumors were considered E- and N-cadherin negative, while moderate and strong cases were regarded positive for these markers.

A similar method was used for podoplanin. Staining intensity was classified as negative (0), weak (1), moderate (2) and strong (3). These values were multiplied by the percentage of cells with cytoplasmic and/or membranous immunostaining, which was calculated as follows: no immunoreactive tumor cells: 0; staining of 1%–10% neoplastic cells: 1; observation of 11%–30% positivity: 2; immunostaining of 51%–50% neoplastic cells: 3; immunoreactivity in 51%–80% tumor cells: 4 and staining of 81%–100% of neoplastic cells: 5. The final scores ranged from 0 to 15 with 0–3, 4–7 and >8 representing weak, moderate and strong staining, respectively. All neoplasms with strong immunoreactivity (final scores >8), were classified as Pod-positive.

A combination of these three markers was used for dividing the SCC specimens into 3 invasion phenotypes as described previously.[3] ECad+/NCad−/Pod+ tumors were considered as having CCI, ECad−/NCad+/Pod− cases were regarded as demonstrating EMT and a combination of all other staining patterns was grouped together as non-EMT/non-CCI.

All analyses were performed by two oral and maxillofacial pathologists under a double-headed microscope and disagreements were resolved by consensus.

2.4. Statistical analysis

Chi-Square was used for statistical analysis and P values of less than 0.05 were considered significant.

3. Results

We retrieved a total of 37 lower lip and 44 oral SCCs from our pathology archive and none of the oral lesions were of the verrucous and exophytic subtypes. According to the immunohistochemical expression profile, the lesions were divided into three groups. The EMT group had a profile of ECad−/NCad+/Pod− that included 5 samples of lower lip SCC (14%) while this pattern was not observed in any of the OSCC cases. The CCI group with an expression pattern of ECad+/NCad−/Pod+, included 12 samples (27%) of OSCC and 4 specimens (11%) of lower lip SCC. The third group consisted of lesions that showed immunohistochemical profiles other than that of EMT and CCI as follows: 15, 3, 12 and 2 oral cavity tumors with ECad−/NCad−/Pod−, ECad−/NCad−/Pod+, ECad+/NCad−/Pod− and ECad+/NCad+/Pod− profiles, respectively. Correspondingly these profiles were found in 14, 5, 6 and 3 lower lip SCCs. In this group there were a total of 32 samples (73%) of OSCC and 28 samples (76%) of lower lip SCC. Figs. 1–3 demonstrate immunoexpression of ECad, NCad and Podoplanin, respectively.

Chi square showed a significant difference in the evaluated invasion patterns between oral cavity and lower lip SCCs (P = 0.009).

4. Discussion

Researchers believe that molecular manifestations of tumors can play a major role in their infiltrative behavior. ECad, NCad, and Pod are markers that have been previously evaluated in the context of cell invasion and were selected to compare the invasive phenotypes of oral and lower lip SCC for the first time in the present study. According to our results, the invasion pattern of SCC

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