Immunolocalization of DNMT1 and DNMT3a in Salivary Gland Neoplasms

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DNA methyltransferase · Methyltransferase · Salivary gland neoplasms · Methylation · Epigenetics

Abstract
Objective: Salivary gland neoplasms pathogenesis has not been well established. DNA methylation occurs when methyl groups are added to cytosine nucleotides in specific areas of the gene by the enzyme DNA methyltransferase (DNMT). This chemical modification can alter gene expression without altering DNA sequence. While DNMT3a is mostly involved in de novo methylation, DNMT1 acts as a maintenance methyltransferase. We aimed to investigate the immunolocalization of DNMT3a and DNMT1 in minor salivary gland neoplasms, comparing it with normal tissue. Material: Forty-four formalin-fixed and paraffin-embedded samples of pleomorphic adenoma, adenoid cystic carcinoma, mucoepidermoid carcinoma and polymorphous low-grade adenocarcinoma were included in the study. The DNMT1 and DNMT3a proteins were identified by using a highly sensitive polymer-based system. Results: Positive nuclear and cytoplasmic labeling for DNMT1 was observed in all samples, including the controls. Positive nuclear labeling for DNMT3a was found only in few neoplasms: 1 pleomorphic adenoma (9.0%), 2 adenoid cystic carcinoma (16.6%) and 1 mucoepidermoid (9.0%) cases. Conclusion: Our results were not able to demonstrate a clear correlation between DNMT1 and DNMT3a immunolocalization in salivary gland neoplasms development.

Introduction
Salivary gland neoplasms are relatively uncommon lesions representing 0.3–1.5% of all biopsies processed in oral pathology laboratories, predominantly affecting major salivary glands [1]. Minor glands account for 9–23% of all salivary gland neoplasms [1]. The intraoral site most commonly affected by benign and malignant neoplasms is the palate. Pleomorphic adenoma (PA) is the most common benign salivary gland neoplasm, while mucoepidermoid carcinoma (MEC) is the most common malignant one, followed by adenoid cystic carcinoma (ACC) and polymorphous low-grade adenocarcinoma (PLGA) [1].

Recent studies have demonstrated that the development and progression of human malignancies are associated with accumulation of alterations in proto-oncogenes and tumor suppressor genes [2, 3]. Epigenetic alterations such as DNA methylation are important for human carcinogenesis, but its role in salivary gland neoplasias has not been well established.
DNA methylation is a phenomenon that occurs when methyl groups are added to cytosine nucleotides in specific areas of the gene by the enzyme DNA methyltransferase (DNMT) [4]. This chemical modification of DNA and its associated proteins can alter gene expression without altering DNA sequence. A number of DNMTs have been identified in mammals. DNMT3a and DNMT3b are mostly involved in de novo methylation, whereas DNMT1 acts as a maintenance methyltransferase [5–7]. Epigenetic silencing of gene expression by promoter CpG island hypomethylation has been shown to be important in the formation of a variety of cancers including oral squamous cell carcinoma [8]. Indeed, several studies have demonstrated hypermethylation events in ACC [9–11], MEC [11–13], PA [11–14], malignant PA [10], salivary duct carcinoma [11] and acinic cell carcinoma [11].

DNMTs were initially found to be overexpressed in tumorigenic cell lines [15] and in a few types of human neoplasms [16–18]. There is scarce information about DNMT1 immunoexpression in MEC, and apparently no data about immunoexpression pattern of DNMT3a in salivary gland neoplasms [12]. In this study, the immunoexpression pattern of DNMT3a and DNMT1 was investigated in benign and malignant minor salivary gland neoplasms, as well as in minor normal salivary glands.

Table 1. Clinical data of the cases of the normal salivary glands, PA, PLGA, adenoid cystic carcinoma and MEC included in the study

<table>
<thead>
<tr>
<th></th>
<th>Mean age, years</th>
<th>Gender</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>female</td>
<td>male</td>
<td>lower lip</td>
</tr>
<tr>
<td>NSG (n = 11)</td>
<td>19 (13–22)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>PA (n = 11)</td>
<td>45 (16–86)</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>PLGA (n = 10)</td>
<td>54 (20–84)</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>ACC (n = 12)</td>
<td>38 (21–85)</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>MEC (n = 11)</td>
<td>45.5 (14–73)</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

Figures in parentheses are ranges. NSG = Normal salivary glands.

Results

All lesions investigated showed broad positive nuclear and cytoplasmic labeling for DNMT1 (grade 2; fig. 1). In the normal salivary glands, positive nuclear staining for DNMT1 was observed in the ductal and acinar cells (grade 2). The immunoexpression pattern of DNMT3a in normal as well as in tumor cells is shown in figure 2. Ductal and acinar cells in normal salivary glands presented only cytoplasmic staining (grade 2). One PA (9.0%) presented nuclear (grade 1) and cytoplasmic labeling (grade 2). Nuclear staining was also observed in 2 ACC (grade 2, 16.6%) and 1 MEC (grade 1, 9.0%; fig. 2). Therefore, only 4 (9.0%) salivary gland neoplasms investigated presented DNMT3a nuclear staining.
Discussion

In the present investigation we examined the immunoreexpression pattern of two catalytically active DNMT in minor salivary gland neoplasms. Overexpression of both DNMT1 and DNMT3a has previously been reported in human neoplasias, including hepatocarcinomas, prostate and breast neoplasms, as well as gastric carcinomas [19–21]. Apparently, no such study has been performed in salivary gland neoplasms.

Hypermethylation of tumor suppressor genes that are normally unmethylated correlates with loss of expression in cancer cells. Hypermethylation of the tumor suppressor genes p16INK4a, p14ARF, p27Kip1, E-cadherin, 14-3-3σ, RASSF1A, DAPK, MGMT, RARβ2 and RB1 has been demonstrated in some cases of salivary gland neoplasms [9–14, 22]. Also, overexpression of various DNMTs in lung cancer may result in promoter hypermethylation of multiple tumor suppressor genes which then leads to poor prognosis [18].
While some studies revealed significant overexpression of DNMT1 in some human neoplastic tissues [15, 18, 23–25], others have found no significant difference of this protein expression in neoplasms when compared to matched normal tissues [26, 27]. Although DNMT1 is considered a maintenance form of DNMT that copies methylation patterns after DNA replication, in human cancers unknown factors could target this enzyme to unmethylated substrate DNA. So some authors believe that DNMT1 protein overexpression could result in de novo DNA hypermethylation during carcinogenesis [25]. Regarding DNMT3a, its expression was shown to be slightly increased in neoplasms from bladder, colon, kidney and pancreas when compared to matched normal tissue [26, 27], and it is suggested that its overexpression may take part in gastric carcinogenesis [28]. In hepatocarcinogenesis, a sequential decrease in cytoplasmic immunoreactivity for DNMT3a and a concurrent increase in nuclear DNMT3a were observed in high-grade dysplasia and carcinomas when compared to nonneoplastic and low-grade dysplasia [29].

All cases included in our study showed a broad nuclear positive immunostaining for DNMT1 in all cell layers, and so did the fragments of normal salivary glands, suggesting that this enzyme may not be relevant to salivary gland tumorigenesis. In addition, only 9% of the salivary gland neoplasms analyzed presented nuclear expression of DNMT3a. Although hypermethylation of tumor suppressor genes is reported in salivary gland tumors [9–14, 22], our data suggests that these epigenetic changes might not be associated with an increased expression of DNMT3a.

In conclusion, our results were not able to demonstrate a clear correlation between DNMT1 and DNMT3a immunoeexpression and salivary gland neoplasms development. The present findings, however, need to be confirmed by studies using other assays.

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References


