INTRODUCTION

The c-kit proto-oncogene (CD117) is a type III transmembrane receptor tyrosine kinase, encoded by the c-kit gene that is located on the human chromosome segment 4q11.[1,2] The loss of function mutations have demonstrated the crucial role of c-kit in normal growth and/or differentiation of several cell types.[3,4] C-kit interaction with its ligand, stem cell factor (SCF, also called steel factor) leads to activation of specific intracellular signal transduction cascades.[5]

The overexpression of c-kit has been implicated in a number of human neoplasms including gastrointestinal stromal tumors, ovarian cancer, testicular germ cell tumors, small and non-small lung cancers, acute myeloblastic leukemia and malignant melanoma, suggesting a role for c-kit and its mutant forms in carcinogenesis.[6-10]

Adenoid cystic carcinoma (AdCC) is a malignant salivary gland neoplasm characterized by indolent growth pattern, a high rate of metastasis with late onset, tendency to invade neural tissue and low rate of long-term survival. Since AdCC exhibits invasive behavior, differentiating this tumor from other benign and malignant salivary gland tumors that share similar histologic features in small biopsies, such as polymorphous low-grade adenocarcinoma (PLGA) and pleomorphic adenoma (PA), is critical.[11,12]

Currently, there is little information on the altered expression of c-kit in salivary gland tumors, which is mainly limited to...
AdCC and PLGA. Furthermore, recent studies about using c-kit for distinguishing AdCC from other salivary gland tumors are controversial. The purpose of this work is to investigate the expression and tissue distribution of c-kit protein in different types of benign and malignant salivary gland tumors that mimic AdCC and to evaluate the application of c-kit as a marker in the diagnosis of AdCC.

MATERIALS AND METHODS

We obtained paraffin-embedded tissue blocks of 36 benign and malignant prevalent tumors including nine AdCC (cribriform type), four PLGA, six mucoepidermoid carcinoma and 17 PA from the files of the Pathology Departments of Hospitals and the Oral and Maxillofacial Pathology Department of Dental School. The paraffin-embedded tissue blocks were sliced into three-micrometer sections for routine histological and subsequent immunohistochemical examinations. Diagnosis of the AdCC, PLGA, MEC and PA was based on histological examination of the hematoxylin-and-eosin-stained tissue sections.

Immunohistochemical staining for c-kit

Immunohistochemistry was performed on formalin-fixed and paraffin-embedded three-micrometer thick sections. Tissue sections were deparaffinized in xylene and rehydrated in decreasing ethanol series. For antigen retrieval, sections were boiled in 0.01 citrate buffer (pH = 6) for ten minutes. Methanol with 0.5% hydrogen peroxide was used to block endogenous peroxidase activity for ten minutes. Tissue sections were then washed in Tris-buffered saline (TBS, pH = 7.6) and incubated with diluted normal serum for ten minutes and then treated with primary antibody for 30 min according to the procedure outlined by the manufacturer. The applied primary antibody was c-kit monoclonal antibody (Novocastra RTU-CD117, RE 7290 k, UK).

After rinsing with TBS, the sections were incubated with secondary antibody, washed again in TBS and reacted with diaminobenzene hydrochloride (DAB) for five minutes. Finally, slides were counterstained with hematoxylin and cover-slipped with a synthetic mounting media. Tissue mast cells and stained sections of gastrointestinal stromal tumors (GIST) were used as positive controls and lack of the primary antibody as negative control.

The immunostains were evaluated by three independent reviewers and the slides were examined with a light microscope at a final magnification of ×400. In this study, only the percentage of cells that showed cytoplasmic or membranous staining, but not the intensity of staining, was quantified.

According to the percentage of the positive cells, immunohistochemical reactivity for c-kit was scored as follows:

| Staining intensity (%) | WEAK | MODERATE | STRONG |
|------------------------|------|----------|--------|
| Normal histopathology  | 10   | 0.0      | 0.0    |
| Pleomorphic adenoma    | 17.6 | 58.8     | 0.0    |
| Adenoid cystic carcinoma | 11.1 | 44.4     | 44.4   |
| Polymorphous low-grade adenocarcinoma | 50 | 25 | 25 |
| Mucoepidermoid carcinoma | 33.3 | 50 | 16.7 |

Statistical analyses

Data were analyzed with PASW Statistics 18 for Windows. Quantitative Assessment of the relationship between variable parameters and comparison of different groups were analyzed with Chi-Square test with P < 0.05 being considered statistically significant.

RESULTS

In case of AdCC, all samples showed the expression of the c-kit protein. In case of approximately 90% of AdCC samples, more than 50% of the cells exhibited positive reactivity for c-kit (graded as 3+) and in one case 10-25% of cells were positive (1+). The highest frequency of the grade 3+ c-kit was observed in AdCC (42.1%). In 88.8% of AdCC cases, staining intensity was moderate or strong (4/9 moderate and 4/9 strong) [Table 1 and Figure 1a and b]. The site of histological manifestation of c-kit was mainly diffuse (7/9 AdCC cases were diffuse and 2/9 cases were luminal) and the cellular localization of c-kit was found to be membranous and/or cytoplasmic (7/9 cases of AdCC were membranous and cytoplasmic and 2/9 cases were merely membranous).

Table 1: Percentage of different staining intensities of c-kit protein in the studied tumors and control samples

![Figure 1](https://example.com/figure1.jpg)
In case of PLGA samples, the c-kit expression was observed in all samples (4/4) with less than 50% of the cells showing positive reactivity for c-kit (3/4 were 2+ [26-50% of the cells] and 1/4 was 1+ [10-25% of the cells]). Furthermore, the staining intensity for c-kit varied from weak to strong [Table 1]. The histological manifestation of c-kit was found to be diffuse (2/4) and luminal (2/4) and c-kit was mainly localized in the cytoplasm (3/4 were cytoplasmic and 1/4 was membranous) [Table 2 and Figure 2a and b].

In case of MEC, similar to AdCC and PLGA samples, c-kit expression was positive in all MEC specimens. The immunoreactivity for c-kit was 3+ in 66.7% of the cases and 2+ or 1+ in 2 cases [Table 3 and Figure 3a and b]. Furthermore, similar to AdCC, the site of histological manifestation of c-kit was diffuse (4/6) [Table 2] and the cellular localization of c-kit in MEC samples predominantly exhibited the cytoplasmic-membranous pattern (4/6) and in only two cases the membranous pattern was observed (2/6).

In contrast to previous tumors, PA samples showed the expression of c-kit in 76.5% (13/17) of cases and thus, negative results for c-kit expression were observed only in PA samples [Table 3 and Figure 4a-c]. Furthermore, the manifestation of c-kit was largely diffuse in the positive samples and c-kit was mostly localized to the membrane (9/13 membranous, 3/13 cytoplasmic and 1/13 both).

All control samples showed no expression of c-kit, except one sample, which showed c-kit immunoreactivity 1+ with a weak staining intensity.

Therefore, while our results showed a significant difference in the expression of c-kit between the control and tumor specimens ($P < 0.05$), no significant difference was observed between the benign (PA) and malignant (AdCC, PLGA, and MEC) tumors.

**DISCUSSION**

The c-kit proto-oncogene protein is a transmembrane receptor-type III tyrosine kinase that shows structural homology to the receptors of platelet-derived growth factor, macrophage colony stimulating factor and Flt3. Upon binding to its ligand, stem cell factor, it begins a signal cascade that contributes to the growth and differentiation of multiple hematopoietic lineages.$^{[13]}$

The c-kit gene product is expressed in several normal cells including mast cells and epithelial cells of breast.

**Table 3: Frequency and percentage of c-kit expression in the studied tumors and control samples**

| Histopathology                       | C-kit | - | + | ++ | +++ |
|--------------------------------------|-------|---|---|----|-----|
| Normal histopathology                | Count | 9 | 1 | 0  | 0   |
|                                      | Percentage | 90.0 | 10.0 | 0.0 | 0.0 |
| Pleomorphic adenoma                  | Count | 4 | 1 | 5  | 7   |
|                                      | Percentage | 23.5 | 5.9 | 29.4 | 41.2 |
| Adenoid cystic carcinoma             | Count | 0 | 1 | 0  | 8   |
|                                      | Percentage | 0.0 | 11.1 | 0.0 | 88.9 |
| Polymorphous low-grade adenocarcinoma| Count | 0 | 1 | 3  | 0   |
|                                      | Percentage | 0.0 | 25.0 | 75.0 | 0.0 |
| Mucoepidermoid carcinoma             | Count | 0 | 1 | 1  | 4   |
|                                      | Percentage | 0.0 | 16.7 | 16.7 | 66.7 |

**Table 2: Percentage of different sites of histological expression of c-kit in the studied neoplasms and control samples**

| Histopathology               | Site of histological expression (%) |
|-----------------------------|-------------------------------------|
|                             | Luminal | Diffuse |
| Normal histopathology       | 0.0%    | 100%    |
| Pleomorphic adenoma         | 23.1%   | 76.9%   |
| Adenoid cystic carcinoma    | 22.2%   | 77.8%   |
| Polymorphous low-grade adenocarcinoma | 50% | 50% |
| Mucoepidermoid carcinoma    | 33.3%   | 66.7%   |

**Figure 2:** (a) Photomicrograph of polymorphous low-grade adenocarcinoma (PLGA) (H&E stain, ×100). (b) C-kit expression in PLGA samples monitored by immunostaining. An immunoreactivity of 2+; strong staining intensity and diffuse expression was observed in PLGA samples (IHC stain, ×40)

**Figure 3:** (a) Photomicrograph of mucoepidermoid carcinoma (MEC) samples (H&E stain, ×100). (b) Immunostaining for c-kit expression in MEC. MEC samples exhibit weak staining intensity (IHC stain, ×100). Inset: High power view of the same (IHC stain, ×400)
role of c-kit in the normal migration and development of germ cells and melanocytes has been demonstrated.\textsuperscript{[3,4]}

Alteration in c-kit expression has been observed in a variety of neoplasms including gastrointestinal stromal cell tumors (GISTs), germ cell tumors and salivary gland tumors.\textsuperscript{[1,12]}

Until recently, a limited number of studies have examined the expression of c-kit protein in salivary glands. Several studies have reported a consistently strong expression of the c-kit protein (CD117) in AdCC. However, some other salivary gland tumors including PA and PLGA may also be immunoreactive, and studies attempting to differentiate PLGA from AdCC have shown discrepant results.\textsuperscript{[14,15]} Indeed, differentiating between AdCC and other salivary gland tumors, especially PLGA, can be a diagnostic challenge. Penner et al. demonstrated that c-kit might be a valuable adjunctive tool for differentiating AdCC from PLGA or benign from malignant neoplasm, whereas Edwards et al. suggested that c-kit was not a useful marker for diagnosis.\textsuperscript{[12]}

In addition to the role of c-kit in the diagnosis of AdCC, the relationship of c-kit expression with clinical findings was also evaluated by Lee et al. They reported that the expression of c-kit had no predictive value for recurrence and prognosis.\textsuperscript{[16]} The discrepancy in the previous results compelled us to investigate the potential of c-kit for the diagnosis of AdCC.

In this study, we explored c-kit protein expression in certain types of neoplastic and non-neoplastic salivary gland specimens. Consistent with previous reports, all cases of AdCC exhibited the expression of c-kit protein in this malignant neoplasm.\textsuperscript{[1,11,12,17-22]}

According to WHO classification, AdCC has three microscopic patterns: Tubular (well-differentiated or grade I), cribriform (moderately differentiated or grade II) and solid pattern (poorly differentiated or grade III).\textsuperscript{[23]} The most common pattern is cribriform and the less frequent one is the solid type. A mixture of patterns is common in AdCC and classification is done according to the predominant pattern.\textsuperscript{[15]} Although the mixed type was also seen in our samples, the cribriform pattern was prominent and thus, our cases were classified as the cribriform type.

In our analyses, most of the AdCC samples showed immunoreactivity for c-kit protein in more than 50% of the cells (3×). Similar observations had previously been reported by Epivatianos et al.,\textsuperscript{[17]} Andreadis et al.,\textsuperscript{[19]} Chandan et al.,\textsuperscript{[21]} and Penner et al.\textsuperscript{[12]} Furthermore, several studies on AdCC have shown c-kit protein expression in neoplastic cells exhibiting solid pattern; luminal cells of tubular structures and cells lining cribriform spaces.\textsuperscript{[11,12,17,19,22]} Notably, in this study, we could demonstrate a diffuse pattern of c-kit expression in AdCC specimens, which can explain the discrepancy in previous observations. We find that in AdCC, the c-kit protein has a cytoplasmic-membranous localization, which concurs with the findings of Mino et al.,\textsuperscript{[11]} and differs from the previous reports by Chandan et al.,\textsuperscript{[21]} Edwards et al.\textsuperscript{[22]} and Jeng et al.,\textsuperscript{[1]} showing only a cytoplasmic localization of c-kit protein.

The role of c-kit pathway in carcinogenesis in AdCC is not yet clear. Oliveira et al. have reported that although c-kit is expressed in AdCC, it is not phosphorylated, suggesting that it is unlikely to have a direct oncogenic function in AdCC.\textsuperscript{[11,24]} The strong expression of c-kit in AdCC may suggest a role for c-kit inhibitors as potential therapeutic drugs for this tumor. Imatinib mesylate (Glivec) is a tyrosine kinase inhibitor (TKI) that inhibits both the platelet-derived growth factor receptor (PDGFR) and the c-kit receptor. It was found to be effective against gastrointestinal stromal tumor (GIST) that harbors a genetic mutation in c-kit. Imatinib has been evaluated in patients with AdCC and objective response was reported only in a limited subset of patients.\textsuperscript{[25-28]} Since the effect of tyrosine kinase inhibitor therapy depends on the presence of mutations associated with c-kit overexpression and the mutation of c-kit is uncommon, the absence of mutation in c-kit may explain why imatinib is less effective on AdCC than on GIST.\textsuperscript{[29]}

In addition to the strong expression of c-kit in AdCC, our results demonstrate that the c-kit protein is also expressed in all PLGA samples. However, according to Andreadis et al.\textsuperscript{[19]} and Penner et al.\textsuperscript{[12]} 50% of their examined PLGA cases showed no expression for c-kit. Furthermore, consistent with previous studies, less than 50% of the cells of PLGA specimens displayed immunoreactivity for c-kit.\textsuperscript{[12,17,19]}

Thus, these results suggest that c-kit cannot be considered as a reliable marker to differentiate between AdCC and PLGA neoplasms.\textsuperscript{[12]} Furthermore, the histological expression...
of c-kit in PLGA was diffuse, which is consistent with the observations of Epivatianos et al. and Andreadis et al.[17,19]

Significantly, in spite of the previous reports by Andreadis et al.[19] Mino et al.[11] and Jeng et al.,[1] showing no expression for c-kit in MEC neoplasm, we found c-kit expression in the majority of our MEC specimens.

In case of PAs, the majority of samples showed immunoreactivity for c-kit, which is consistent with the findings of Andreadis et al.[19] and Chandan et al.[21] In comparison with AdCC and PLGA, PA samples showed varied immunoreactivity for c-kit from 1+ to 3+, which is in line with the study of Chandan et al.[20] Although, in previous study by Andreadis et al.[19] on PA, only the luminal cells of the duct-like structures showed positive staining for c-kit (while the solid tumor cells and reticular or trabecular areas were negative), we found diffuse distribution of c-kit protein in PA. Furthermore, we found that in PA, c-kit is primarily expressed in the cell membrane, which is in agreement with the findings of Andreadis et al.[19] and contrary to previous report by Chandan et al.,[21] where the c-kit protein was found in the cytoplasm of tumor cells.

Taken together, we demonstrate that c-kit expression is not restricted to AdCCs and is rather displayed in other benign and malignant neoplasms such as PA, PLGA and mucoepidermoid carcinoma. Albeit several reports[11,12,17,18] have suggested the expression of c-kit protein as a useful marker in differential diagnosis of AdCCs from other types of salivary gland neoplasms, particularly in small biopsy specimens, our study together with the studies of Chandan et al.[21] and Edwards et al.[22] strongly oppose previous conclusions.

CONCLUSION

Our results demonstrate that c-kit expression is not only limited to AdCC among other salivary gland tumors and thus, it cannot be considered as a diagnostic marker for differentiation of AdCC from other benign and malignant salivary gland neoplasms.

Furthermore, these results failed to detect significant difference in the c-kit expression between benign and malignant tumors and thus it cannot be used for differential diagnoses between these two types of salivary gland neoplasms. Notably, percentage of the cells of AdCC samples immunostained for c-kit was higher than 50% whereas, in PLGA samples, less than 50% of tumor cells showed immunoreactivity for c-kit. Indeed, the percentage of immunoreactivity of the tumor cells for c-kit could potentially be an important factor in differentiating AdCC from PLGA. Moreover, to distinguish between AdCC and PA, analysis of staining intensity for c-kit, rather than c-kit expression itself, should be preferentially utilized (i.e., strong staining intensity for c-kit is merely observed in AdCC samples).

Further investigation is required to characterize c-kit functional pathways in salivary gland tumors and to evaluate potential therapeutic effects of small molecule inhibitors of c-kit on these tumors.

ACKNOWLEDGEMENT

The results given in this paper were obtained from a postdoctoral research supported by the Vice Chancellor of Mashhad University of Medical Sciences.

REFERENCES

1. Jeng YM, Lin CY, Hsu HC. Expression of the c-kit protein associated with certain subtypes of salivary gland carcinoma. Cancer Lett 2000;154:107-11.
2. Yarden Y, Kuang WJ, Yang-Feng TJ, Coussens L, Munemitsu S, Dull TJ, et al. Human proto-oncogene c-kit: A new cell surface receptor tyrosine kinase for an unidentified ligand. EMBO J 1987;6:3341-51.
3. Kierszenbaum AL. Tyrosine protein kinases and spermatogenesis: Truncation matters. Mol Reprod Dev 2006;73:399-403.
4. Wang MC, Bohmann D, Jasper H. JNK signaling confers tolerance to oxidative stress and extends lifespan in Drosophila. Dev Cell 2003;5:811-7.
5. Martinon F, Burns K, Tschopp J. The inflammasome: A molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell 2002;10:417-26.
6. Guerriere-Kovach PM, Hunt EL, Patterson JW, Glembocki DJ, English JC 3rd, Wick MR. Primary melanoma of the skin and cutaneous melanomatous metastases: Comparative histologic features and immune phenotypes. Am J Clin Pathol 2004;122:70-7.
7. Leroy X, Augusto D, Leteurtre E, Gosselin B. CD30 and CD117 (c-kit) used in combination are useful for distinguishing embryonal carcinoma from seminoma. J Histochem Cytochem 2002;50:283-5.
8. Hayat MA. Immunohistochemistry and in situ hybridization of human carcinomas. Vol. 1 th ed. San Diego: Elsevier; 2004.
9. Sekido Y, Obata Y, Ueda R, Hida T, Suyama M, Shimokata K, et al. Preferential expression of c-kit protooncogene transcripts in small cell lung cancer. Cancer Res 1991;51:2416-9.
10. Sarlomo-Rikala M, Kovatich AJ, Barbusевич A, Miettinnen M. CD117: A sensitive marker for gastrointestinal stromal tumours that is more specific than CD34. Mod Pathol 1998;11:728-34.
11. Mino M, Pilch BZ, Faquin WC. Expression of KIT (CD117) in neoplasms of the head and neck: An ancillary marker for adenoid cystic carcinoma. Mod Pathol 2003;16:1224-31.
12. Penner CR, Folpe AL, Budnick SD. C-kit expression distinguishes salivary gland adenoid cystic carcinoma from polymorphous low-grade adenocarcinoma. Mod Pathol 2002;15:687-91.
13. Vandenbark GR, deCastro CM, Taylor H, Dew-Knight S, Kaufman RE. Cloning and structure analysis of the human c-kit gene. Oncogene 1992;7:1259-66.
14. Premkumar J, Karthik S, Sathyakumar M, Martin Y Concurrent occurrence of adenoid cystic carcinoma of the salivary glands with small cell carcinoma of the liver: A rare case report. J Oral Maxillofac Pathol 2013;17:288-91.
15. Gnepp DR. Diagnostic surgical pathology of head and neck. 2nd ed. Philadelphia: WB Saunders Co; 2009.
C-kit expression in salivary gland neoplasms

Salehinejad, et al. 182

16. Lee SK, Kwon MS, Lee YS, Choi SH, Kim SY, Cho KJ, et al. Prognostic value of expression of molecular markers in adenoid cystic cancer of salivary glands compared with lymph node metastasis: A retrospective study. World J Surg Oncol 2012;10:266.

17. Epivatianos A, Poulopoulos A, Dimitrakopoulos I, Andreadis D, Nomikos A, Vlahou S, et al. Application of alpha-smooth muscle actin and c-kit in the differential diagnosis of adenoid cystic carcinoma from polymorphous low-grade adenocarcinoma. Oral Oncol 2007;43:67-76.

18. Beltran D, Faquin WC, Gallagher G, August M. Selective immunohistochemical comparison of polymorphous low-grade adenocarcinoma and adenoid cystic carcinoma. J Oral Maxillofac Surg 2006;64:415-23.

19. Andreadis D, Epivatianos A, Poulopoulos A, Nomikos A, Papazoglou G, Antoniades D, et al. Detection of C-KIT (CD117) molecule in benign and malignant salivary gland tumours. Oral Oncol 2006;42:57-65.

20. Freier K, Flechtenmacher C, Walch A, Devens F, Mühlung J, Lichter P, et al. Differential KIT expression in histological subtypes of adenoid cystic carcinoma (ACC) of the salivary gland. Oral Oncol 2005;41:934-9.

21. Chandan VS, Wilbur D, Faquin WC, Khurana KK. Is c-kit (CD117) immunolocalisation in cell block preparations useful in the differentiation of adenoid cystic carcinoma from pleomorphic adenoma? Cancer 2004;102:207-9.

22. Edwards PC, Bhuia T, Kelsch RD. C-kit expression in the salivary gland neoplasms adenoid cystic carcinoma, polymorphous low-grade adenocarcinoma, and monomorphic adenoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:586-93.

23. Seifert G, Sobin LH. Histological typing of salivary gland tumours. 2nd ed. New York: Springer-Verlag; 1991.

24. Oliveira AM, Hornick JL, Duensing A, Medeiros F, Fletcher CD, Fletcher JA. KIT expression and activation in adenoid cystic carcinoma. Mod Pathol 2003;16:221A.

25. Hotte SJ, Winquist EW, Lamont E, Mackenzie M, Vokes E, Chen EX, et al. Imatinib mesylate in patients with adenoid cystic cancers of the salivary glands expressing c-kit: A Princess Margaret Hospital phase II consortium study. J Clin Oncol 2005;23:585-90.

26. Guigay J, Bidault F, Temam S, Janot F, Raymond E, Faivre S. Antitumor activity of imatinib in progressive, highly expressing kit adenoid cystic carcinoma of the salivary glands: A phase II study. J Clin Oncol 2007;25.

27. Pfeffer MR, Talmi Y, Catane R, Symon Z, Yosepovitch A, Levitt M. A phase II study of imatinib for advanced adenoid cystic carcinoma of head and neck salivary glands. Oral Oncol 2007;43:33-6.

28. Alcedo JC, Fabrega JM, Arosemena JR, Urrutia A. Imatinib mesylate as treatment for adenoid cystic carcinoma of the salivary glands: Report of two successfully treated cases. Head Neck 2004;26:829-31.

29. McGurk M, Cascarini L. Controversies in the management of salivary gland disease. 2nd ed. Oxford University Press; 2013.

How to cite this article: Salehinejad J, Mohtasham N, Bagherpour A, Abbaszadeh-bidokhty H, Ghazi A. Evaluation of c-kit protein (CD117) expression in common salivary gland neoplasms. J Oral Maxillofac Pathol 2014;18:177-82.

Source of Support: : The results given in this paper were obtained from a postdoctoral research supported by the Vice Chancellor of Mashhad University of Medical Sciences, Iran. Conflict of Interest: None declared.