

IMMUNOHISTOCHEMICAL EXPRESSION OF C-KIT (CD117) PROTIEN IN SALIVARY GLAND TUMORS

Thesis

**Submitted to the Faculty of Oral and DentalMedicine,
Cairo University, in Partial Fulfillment of the Requirements for
The Degree of Master of Dental Science
(ORAL PATHOLOGY)**

BY

EMAN ALI KASEM AGHA

B.D.S. of Dental Science (Tripoli University)

2003

**Department of Oral Pathology
Faculty of Oral and Dental Medicine
Cairo University**

2012

Supervisors

Prof. Dr.SamiaMustafa El-Azab

Professor of Oral Pathology

Faculty of Oral and Dental Medicine

Cairo University.

Dr.SafaFathyAbd El-Ghani

Lecturer of Oral Pathology

Faculty of Oral and Dental Medicine

Cairo University.

TO my family

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



صدق الله العظيم

Acknowledgement

I am deeply grateful to Dr. *Samia El-azab*, Professor of Oral Pathology, Faculty of Oral and Dental Medicine, Cairo University, for her wise guidance, valuable advice, close supervision and constant encouragement throughout this work.

I would like also to express my sincere gratitude to Dr. *Safa Fathy AbdElg-ghani*, Lecturer of Oral Pathology, Faculty of Oral and Dental Medicine, Cairo University, for her faithful assistance, co-operation, patience and consistent advice.

Finally, I wish to thank all the staff members of Oral Pathology Department, Faculty of Oral and Dental Medicine, Cairo University for their help and facilities they offered during the course of study.

Finally, my hearty thanks to the soul my mother, to my father, and my husband for his enthusiastic help, support and love.

Eman Ali Agha
2012

<u>Contents</u>	<u>Pages</u>
Introduction	<u>1</u>
Review of literature	
-Salivary Gland Neoplasms.	<u>2</u>
-Receptor Tyrosine Kinase.	<u>12</u>
-C-Kit.	<u>19</u>
-Immunohistochemical Expression Of C-kit.	<u>28</u>
Aim of the study	<u>33</u>
Material and Method	<u>34</u>
Results	
-Pleomorphic adenoma	<u>46</u>
-Warthin's tumor	<u>57</u>
-Mucoepidermoid carcinoma	<u>61</u>
-Adenoid cystic carcinoma	<u>69</u>
- Statistical Analysis	<u>84</u>
Discussion	<u>94</u>
Conclusion	<u>101</u>
English Summary	<u>102</u>
References	<u>104</u>
Arabic Summary	

List of tables

<i>Number of table</i>	<i>Title</i>	<i>Page</i>
(1)	Classification of SGNs.	3
(2)	A list of the most popular RTKs classes or subfamily.	12
(3)	Clinical data and diagnosis of studied case.	34-35
(4)	Summary of clinical data of studied cases.	46
(5)	Area percentage of c-kit positive cells in all tested SGNs.	86
(6)	Tukey multiple comparasion test of area percentage in all tested SGNs.	86
(7)	Immunostaining intensity of c-kit positive cells in all tested SGNs.	87
(8)	Tukey multiple comparisons test of immunostaining intensity in all tested SGNs.	87
(9)	Area percentage of c-kit positive cells in benign and malignant tumors.	88
(10)	Immunostaining intensity of c-kit positive cells in benign and malignant tumors.	89
(11)	Area percentage of c-kit in different grade of MEC.	90
(12)	Immunostaining intensity of c-kit in different grade of MEC.	91
(13)	Area percentage of c-kit positive cells in different histological patterns of ACC.	92
(14)	Tukey multiple comparisons test of area percentage in ACC patterns.	93
(15)	Immunostaining intensity of c-kit positive cells in different histological patterns of ACC.	94
(16)	Tukeymultiple comparisons test of immunostaining intensity in histological pattern ofACC.	94

List of Figure

<i>Number of FIGURE</i>	<i>Figure legend</i>	<i>Page</i>
(1)	Protein phosphorylation.	13
(2)	Structure of RTKs.	14
(3)	Activation of RTKs.	15
(4)	Different mechanism of RTKs signals attenuation.	17
(5)	C-kit structure	20
(6)	Binding of CSF to c-kit.	22
(7)	Overview of signaling pathways.	23
(8)	Expression of c-kit in hematopoietic cell.	25
(9)	A Photograph of the Image J software (NIH, version v1.45e, USA) analyzer computer system.	43
(10)	A Copy of display seen on the monitor of the image analyzer showing the measurement of optical density of c-kit immunoreactivity in SGNs (X200). The brown immunostain colour converted into grey (A), then masked by a red binary colour (B).	44
(11)	A photomicrograph of a case of PA showing variable thickness of capsule (arrow), duct like structures containing mucin (yellow arrows) and epithelial masses, H&E, X100.	48
(12)	A photomicrograph of a case of PA showing a C.T. capsule with variable completeness, thickness and density. Epithelial masses and myxoid tissue (arrow) are also seen, H&E, X100.	49
(13)	A photomicrograph of PA revealing chondroid tissue (C), duct like structures containing mucin (arrow) and epithelial masses, H&E, X100.	49

<i>Number of FIGURE</i>	<i>Figure legend</i>	<i>Page</i>
(14)	A photomicrograph of PA demonstrating keratin pearls (K), many duct like structures containing mucin (arrows), H&E, X100.	50
(15)	A photomicrograph of PA showing chondroid tissue (C) with few ducts like structures (arrow). Epithelial masses are forming of polyhedral and spindle shaped cells are also observing. Note the stellate cells forming myxoid tissue (M), H&E, X200.	50
(16)	A photomicrograph of a case of PA demonstrating duct like structure (D) and myxoid tissue (M). fibroid tissue also is detecting (arrow), H&E, X400.	51
(17)	A photomicrograph of a case of PA showing duct like structures forming of double layers of cells. One of them containing mucin, H&E, X 400.	51
(18)	A photomicrograph of a case of PA demonstrating heterogeneous moderate to strong cytoplasmic expression of c-kit in duct like structures. Few cells are showing positive nuclear reaction (red arrows), c-kit antibody, X200.	52
(19)	A photomicrograph of a case of PA showing heterogenous moderate to strong cytoplasmic and membranous c-kit immunoreactivity in epithelial masses. Few stellate cells in the myxoid tissue are showing both nuclear and cytoplasmic reaction (arrow), c-kit antibody, X200.	53
(20)	A photomicrograph of a case of PA revealing moderate to strong heterogeneous c-kit immunoreactivity in epithelial cells of duct like structures, mucin showing positive reaction in some ducts. Myxoid tissue is showing negative immunexpression, c-kit antibody, X200.	53
(21)	A photomicrograph of a case of PA demonstrating stronger c-kit immunoreactivity in inner epithelial cells than outer cells (black arrow). Keratin shows positive reaction (yellow arrow), c-kit antibody, X200.	54
(22)	A photomicrograph of a case of PA showing strong cytoplasmic c-kit immunoreactivity in cells of epithelial masses and duct like structures, c-kit antibody, X200.	54

<i>Number of FIGURE</i>	<i>Figure legend</i>	<i>Page</i>
(23)	A photomicrograph of a case of PA revealing strong cytoplasmic c-kit immunoreactivity in epithelial cells forming epithelial masses or lining duct like structures. Note positive cytoplasmic reaction in cells within the chondroid tissue, c-kit antibody, X400.	55
(24)	A photomicrograph of a case of PA demonstrating moderate cytoplasmic expression of c-kit mostly in the inner layer of duct like structure, mucin reaction is detecting, c-kit antibody, X 400.	55
(25)	A photomicrograph of a case of PA showing moderate cytoplasmic expression of c-kit mostly in the inner layer of duct like structure, mucin reaction was detected. Cytoplasmic reaction was observing instellate cells of myxoid tissue, c-kit antibody, X 400.	56
(26)	A photomicrograph of a case of Warthin's tumor demonstrating multiple cystic spaces containing eosinophilic material(green arrow). Double epithelial cell layers (black arrow), lymphoid tissue is observing (yellow arrow), H&E, X200.	57
(27)	A photomicrograph of a case of Warthin's tumor revealing double epithelial cell layers from the inner. Tall columnar cells with granular eosinophilic cytoplasm and darkly stained nuclei. A more cuboidal outer layer of cells with prominent nuclei was seen, H&E, X400.	58
(28)	A photomicrograph of a case of Warthin's tumor showing lymphoid tissue stroma forming a germinal center (G), H&E, X 400.	58
(29)	A photomicrograph of a case of Warthin's tumor demonstrating homogenous strong cytoplasmic c-kit expression in both layers of epithelial lining. All the cells in the lymphoid tissue are showing negative immunoreactivity (red arrow), c-kit antibody, X200.	60

<i>Number of FIGURE</i>	<i>Figure legend</i>	<i>Page</i>
(30)	A photomicrograph of a case of Warthin's tumor showing cytoplasmic c-kit expression in all cells lining the cystic spaces. The lymphoid tissue stroma is totally negative (arrow), c-kit antibody, X200.	60
(31)	A photomicrograph of a case of low grade MEC showing masses of mucous cells (arrow), mucin pools, duct like structures and few collagen fiber bundles, H&E, X100.	62
(32)	A photomicrograph of a case of low grade MEC demonstrating masses of mucous cells with variable sizes and shapes. They are forming of abundant foamy cytoplasm with eccentric nuclei. Epidermoid cells are observing between the mucous cells (arrow), H&E, X 200.	62
(33)	A higher magnification of the previous case revealing mucous cells with foamy cytoplasm and eccentric nuclei. Few epidermoid cells are interposing between mucous cells are also observing, H&E, X400.	63
(34)	A photomicrograph of case of high grade MEC showing sheets of epidermoid (blue arrow) and clear cells (red arrow), H&E, X200.	63
(35)	A photomicrograph of a case of high grade MEC demonstrating solid sheets of epidermoid cells. The cells are showing hyperchromatic nuclei, prominent nucleoli, in addition to cellular and nuclear pleomorphism, H&E, X200.	64
(36)	Another case of high grade MEC revealing solid sheets of epidermoid cells. The cells show hyperchromatic nuclei, prominent nucleoli, in addition to cellular and nuclear pleomorphism, mitotic figure can be also detecting, H&E, X400.	64

<i>Number of FIGURE</i>	<i>Figure legend</i>	<i>Page</i>
(37)	A photomicrograph of a case of low grade MEC showing mild to moderate cytoplasmic c-kit immunoreactivity in mucus cells, and strong immunoreactivity in epidermoid cells are interposing between masses of mucous cells (arrow), c-kit antibody, X200.	66
(38)	A photomicrograph of a case of low grade MEC showing moderate c-kit immunoreactivity in mucous cells, the mucin is showing positive reaction as well (arrow). Note strong expression in epidermoid cells, and positive reaction in collagen fibers, c-kit antibody, X200.	67
(39)	A higher magnification of the previous case showing moderate c-kit immunoreactivity in mucous cells, and strong expression in epidermoid cells. Note few nuclei are showing positive immnnoreaction (arrow), c-kit antibody, X400.	67
(40)	A photomicrograph of high grade MEC demonstrating homogenous strong cytoplasmic c-kit immunoexpression in sheets of epidermoid cells. Some inflammatory cells are showing positive reaction (red arrow) others showing negative reaction (yellow arrow), c-kit antibody, X200.	68
(41)	A higher magnification of the previous case demonstrating strong heterogeneous cytoplasmic c-kit immunoexpression in epidermoid cells. The nuclei are showing negative reaction (arrow), ckit antibody, X400.	68
(42)	A photomicrograph of a case of cribriform pattern of ACC showing numerous cystic spaces containing mucinous or hyalinized material (arrow). These spaces are surrounding by basaloid cells organizing in units these units are separating from each other by connective tissue stroma, H& E, X100.	71
(43)	A photomicrograph of another case of cribriform of ACC revealing multiple cystic spaces are separating from each other by thin intercommunicating rows of basaloid cells. H&E, X200.	71

<i>Number of FIGURE</i>	<i>Figure legend</i>	<i>Page</i>
(44)	Another case of cribriform pattern of ACC showing multi cystic spaces are containing mucinous material, (black arrow). The lining basaloid cells are large with deeply stained nuclei (green arrows), H&E, X400.	72
(45)	A photomicrograph of a case of tubular pattern of ACC showing tubular or ducts like structures which is containing eosinophilic hyaline material (arrow). These structures are separating from each other by hyalinizing connective tissue stroma, H&E, X200.	72
(46)	A photomicrograph of another case of tubular pattern of ACC revealing multiple duct like structures are forming of double layers of cells. While the inner cells are cuboidal, the outer ones are flat cells with oval nuclei representing myoepithelial cells (arrow), H&E, X200.	73
(47)	A higher magnification of the pervious case showing duct like structures are forming of double layers of cells. It is containing eosinophilic mucinous material (arrow), H&E, X400.	73
(48)	Another case of tubular pattern of ACC showing duct like structures forming of double layers of cells. Inner cuboidal cells with deeply stained nuclei (black arrow) and outer flat cells (red arrow) are observing. The cystic spaces are containing eosinophilic material, H&E, X400.	74
(49)	A photomicrograph of a case of trabecular pattern of ACC showing strands of basaloid cells are separating from each other by eosinophilic hyaline ribbons of basement membrane like material, H&E, X100.	74
(50)	A photomicrograph of a case of trabecular pattern of ACC showing strands of basaloid cells, separating from each other by eosinophilic hyaline material, H&E, X200.	75
(51)	A higher magnification of the previous case showing strands of basaloid cells are containing deeply stained nuclei, separating from each other by eosinophilic hyaline material, H&E, X400.	75
(52)	A photomicrograph of solid pattern of ACC revealing regular or irregular discrete compact sheets of tumor cells with different sizes. They are separating from each other by connective tissue stroma, H&E, X 100.	76

<i>Number of FIGURE</i>	<i>Figure legend</i>	<i>Page</i>
(53)	A higher magnification of previous case showing masses of basaloid cells are separating by scanty collagen fiber bundles, H&E, X200.	76
(54)	A higher magnification of the previous case showing nuclear hyperchromatism (yellow arrows), nuclear and cellular pleomorphisim, H&E, X400.	77
(55)	A photomicrograph of a case of cribriform pattern of ACC showing moderate cytoplasmic c-kit expression in most basaloid cell around cystic spaces, c-kit antibody, X200.	79
(56)	A photomicrograph of a case of cribriform pattern of ACC showing moderate cytoplasmic c-kit expression in tumor cells. There is nuclear reaction in some cells (arrows), c-kit antibody, X400.	79
(57)	A photomicrograph of trabecular pattern of ACC showing moderate to strong cytoplasmic c-kit expression in basaloid cells, c-kit antibody, X200.	80
(58)	A photomicrograph of trabecular pattern of ACC showing moderate to strong cytoplasmic c-kit immunoexpression in basaloid cells. Hyaline ribbons of connective tissue stroma are demonstrating positive reaction also, c-kit antibody, X400.	80
(59)	A higher magnification of the previous case showing moderate to strong cytoplasmic c-kit expression in basaloid cells surrounding the hyaline material, c-kit antibody, X400.	81
(60)	A photomicrograph of tubular pattern of ACC showing moderate cytoplasmic c-kit immunostaining in most of the tumor cells, c-kit antibody, X200.	81
(61)	A photomicrograph of another case of tubular pattern of ACC showing moderate cytoplasmic c-kit immunoreaction in tumor cells forming duct like structures, c-kit antibody, X 400.	82
(62)	A photomicrograph of solid pattern of ACC demonstrating strong homogenous c-kit immunostaining in all tumor cells forming sheets. The connective tissue stroma is showing negative immunoreactivity, c-kit antibody, X100.	82
(63)	A photomicrograph of another case of solid pattern of ACC showing homogenous strong cytoplasmic and membranous c-kit expression in all basaloid cells, c-kit antibody, X200.	83

<i>Number of FIGURE</i>	<i>Figure ligand</i>	<i>Page</i>
(64)	A higher magnification of same case showing homogenous strong cytoplasmic and membranous c-kit expression in all basaloid cells. As well as nuclear reaction was observed, c-kit antibody, X400.	83
(65)	A photomicrograph showing negative control of a case of cribriform pattern of ACC, X200	84
(66)	A photomicrograph of uninvolved salivary gland tissue demonstrating strong to moderate c-kit immunoreexpression in ductal cells. The acinar cells are showing moderate cytoplasmic expression. The connective tissue stroma showing weak reaction. C-kit antibody, X200.	84
(67)	A bar chart demonstrating mean area percentage of c-kit immunostaining in all tested SGNs.	86
(68)	A bar chart illustrating mean immunostaining intensity of c-kit among all tested SGNs.	87
(69)	A bar chart representing mean area percentage of c-kit in benign and malignant SGNs.	88
(70)	A bar chart representing mean immunostaining intensity of c-kit in benign and malignant SGNs.	89
(71)	A bar chart illustrating mean area percentage of c-kit in different grades of MEC.	90
(72)	A bar chart demonstrating mean immunostaining intensity of c-kit in different grades of MEC.	91
(73)	A bar chart representing mean area percentage of c-kit among different histological patterns of ACC (CB: Cribriform, TUB: Tubular, TRB: Trabecular, S: Solid).	93
(74)	A bar chart demonstrating mean immunostaining intensity of c-kit among different histological patterns of ACC (CB: Cribriform, TUB: Tubular, TRB: Trabecular, S: Solid).	94