Immunohistochemical Profile of CD44 and RANK in Ameloblastoma and Keratocystic Odontogenic Tumor

A thesis submitted to Faculty of Dentistry -Ain Shams University in partial fulfillment of the requirement for the Master Degree in Oral Pathology

By

Amal Shehab El-Din Shafiaa

B.D.S. Ain Shams University, 2007

SUPERVISORS

Dr. Mohamed Salah El-Din Ayoub

Professor of Oral Pathology

King Abdulaziz University and Ain Shams University

Dr. Mohamed Hossam El-Din El-Malahy

Professor of Oral Pathology

Faculty of Dentistry- Ain Shams University

التعبير المناعى الهستوكيميائى لظهور ال سى دى 44 و رانك في الورم المينائي والأكياس الكيراتينية سنية المنشأ

رسالة مقدمة لكلية طب الأسنان جامعة عين شمس ضمن متطلبات الحصول على درجة الماجستير في علم أمراض الفم

مقدمة من

أمل شهاب الدين شفيع عبدالباسط

بكالوريوس طب القم والاسنان

جامعة عين شمس

2007

المشرفون

أ.د محمد صلاح الدين أيوب

أستاذ بقسم أمراض الفم كلية طب الأسنان - جامعة عين شمس

أ.د. محد حسام الدين الملاحى

أستاذ بقسم أمراض الفم كلية طب الأسنان - جامعة عين شمس

Acknowledgment

After praise ALLAH, The All-Knowing, for enabling me to conduct this study, I would like to acknowledge **Prof. Dr. Mohamed Salah El-Din Ayoub** for his professional supervision and for his pedagogical guidance. His commitment, total devotion to check the updates, and his excellent expert eye for details have chiefly allowed this study to be that coherent.

My acknowledgment extends equally to **Prof. Dr. Mohammad Hossam El-Din El-Malahy** for his professional contribution and for his lenient supervision.

Also, I would like to show my deep appreciation to *Prof. Dr Zeinab Darwish and Dr bacemAbdallah* for providing me cases of ameloblastoma with basaloid, granular and adenoid patterns.

My real gratitude and acknowledgments are also expressed to my lovely mum for encouraging me to perfect my job in this dissertation.

Table of Contents

List of Abbreviations	i
List of Figures	iii
List of Tables	vii
Introduction and Review of Literature	1
Ameloblatoma	3
keratocystic Odontogenic tumor	7
CD44	10
RANK	19
Aim of the study	27
Material and methods	28
Case selection	28
Histopathological Examination	28
Immunohistochemical Examination	33
Statistical Analysis	35
Results	36
Histopathological results	36
Immunohistochemical Results	40
Statistical Results	63
Discussion	67
Conclusions and Recommendation	74
Summary	75
References	77
Arabic summary	98

List of Abbreviations

Am : Ameloblastoma

CD44 : Cluster Differentiation 44

CD44-HA: CD44-Hyaluronic Acid

CD44V : Cd 44 Variant

CSc : Cancer Stem Cells

DAB : Diamino Benzidine

DCs : Dentritic Cells

ECM: Extracellular Matrix

EGF: Epidermal Growth Factor

EMT : Epithelial-Mesenchymal Transition

ERM: Ezrin, Radixin and Meosin

FGFR2: Fibroblast Growth Factor Receptor 2

GEMS: Glucolipid Enriched Membrane Microdomains

HCAM: Homing Cell Adhesion Molecule

HNSCC: Human Head and Neck Carcinoma

KAM : Keratoameloblstoma

KCOT : Keratocystic Odontogenic Tumor

LPS : Lipopolysaccride

MAM: Mural Ameloblatoma

MAPK: Mitogen-Activated Protein Kinase

MAPKs : Mitogen-Activated Protein Kinase

MMP-2 : Matrix Metalloproteinase

MVD : Microvessal Density

NBCCS: Nasel Basel Cell Carcinoma

OKCs : Odontogenic Keratocysts

OPCs : Osteoclast Precursor Cells

OPG : Osteoprotegerin

OPN : Osteopontin

PAM: Peripheral Ameloblastoma

PBS: Phosphate Buffer Saline

RANK: Receptor Activated of Nuclear factor KAPPA-B

RANK-L: Receptor Activated Of Nuclear Factor KAPPA-B_Ligand

RAS : Rat Sarcoma

RHAMM: Receptor for Hyaluronan Mediated Motility

SHH : somic hedgehog pathway

TGF-B1 : Transforming Growth Factor B1

TNF : Tumor Necrosis Factor

TRAF6: Tumor Necrosis Factor Receptor-Associated Factors

VEGF: Vascular Endothelial Growth Factor

WHO : World Health Organization

List of Figures

No.	Legend	Page
Figure 1	CD44 standard and variant exons	14
Figure 2	RANK signaling pathways.	24
Figure 3	Osteoimmunology and RANKL-RANK-OPG.	25
Figure 4	Refined Photomicrograph showing a homogenous	
	membranous KCOT reactivity for CD44.	34
Figure 5	The highest immunoreactivity was selected.	34
Figure 6	The 8-bit gray image is automated to the optimal	
	threshold for measuring the area fraction.	35
Figure 7	Control slide shows negative reaction for CD44.	
	(Original magnification ×20)	40
Figure 8	Control normal slide shows negative reaction for	
	CD44. (Original magnification ×40)	40
Figure 9	Photomicrograph showing a follicular AM. (CD44,	
	Original magnification ×20)	41
Figure 10	Higher magnification of follicular AM (CD44,	
	Original magnification ×40)	41
Figure 11	Photomicrograph showing a plexiforma AM. (CD44,	
	Original magnification ×20)	42
Figure 12	Higher magnification of plexiform AM (CD44,	
	Original magnification ×40)	42
Figure 13	Photomicrograph showing a plexiforma AM (CD44,	
	Original magnification ×40)	43
Figure 14	Photomicrograph showing a unicystic AM (CD44,	
	Original magnification ×20)	44
Figure 15	Higher magnification of unicystic AM (CD44,	
	Original magnification ×40)	44
Figure 16	Photomicrograph showing a unicystic AM, with a	
	characteristic budding of the rete pegs. (CD44,	
	Original magnification ×40)	45
Figure 17	Photomicrograph showing acanthomatous AM	
	(CD44, Original magnification ×20)	46

No.	Legend	Page
Figure 18	Higher magnification of acanthomatous AM (CD44,	
O	Original magnification ×40)	46
Figure 19	Photomicrograph showing a follicular AM (RANK,	
	Original magnification x40)	47
Figure 20	Photomicrograph showing a follicular AM. (RANK,	
	Original magnification x40)	47
Figure 21	Photomicrograph showing a plexiform AM. (RANK,	
	Original magnification x20)	48
Figure 22	Higher magnification of plexiform AM. (RANK,	
	Original magnification x40)	48
Figure 23	Photomicrograph showing a plexiform AM (RANK,	
	Original magnification x40)	49
Figure 24	Photomicrograph showing an adenoid AM with	
	strong homogenous cytoplasmic and nuclear labeling	
	for RANK. (Original magnification x20)	50
Figure 25	Higher magnification of adenoid AM (RANK,	
	Original magnification x40)	50
Figure 26	Photomicrograph showing an acanthomatous AM	
	(RANK, Original magnification x40)	51
Figure 27	Photomicrograph showing an acanthomatous AM	
	(RANK, Original magnification x20)	52
Figure 28	Higher magnification of acanthomatous AM (RANK,	
	Original magnification x40)	52
Figure 29	Photomicrograph showing a granular AM (RANK,	
	Original magnification x20)	53
Figure 30	Higher magnification of granular AM. (RANK,	
	Original magnification x40)	53
Figure 31	Control normal slide shows negative reaction for	
	RANK (Original magnification x20)	54
Figure 32	Control normal slide shows negative reaction for	
	RANK (Original magnification x40)	54
Figure 33	Photomicrograph showing a parakeratinized KCOT.	
	(CD44, Original magnification x20)	55

No.	Legend	Page
Figure 34	Higher magnification demonstrating a para-	
O	keratinized KCOT with subepithelial separation	
	(CD44, Original magnification x40)	55
Figure 35	Photomicrograph showing a parakeratinized KCOT	
	(CD44, Original magnification x40)	56
Figure 36	Photomicrograph showing a parakeratinized KCOT	
	(CD44, Original magnification x40)	57
Figure 37	Photomicrograph showing a parakeratinized KCOT	
	(RANK, Original magnification ×20)	58
Figure 38	Higher magnification of a parakeratinized KCOT	
	with subepithelial hyalinization, thick keratin layer,	
	and inflammatory infiltrates. (RANK, Original	
	magnification ×40)	58
Figure 39	Photomicrograph showing a parakeratinized KCOT	
	(RANK, Original magnification ×20)	59
Figure 40	Higher magnification of parakeratinized KCOT with	
	subepithelial hyalinization, and thick keratin layer. It	
	reveals a strong homogenous cytoplasmic labeling	
	for RANK in the epithelial wall of KCOT. Stromal	
	cells reveal focally weak nuclear immunoreactivity	
	for RANK (Original magnification ×40)	59
Figure 41	Photomicrograph showing a parakeratinized KCOT.	
	(RANK, Original magnification ×20)	60
Figure 42	Photomicrograph showing a parakeratinized KCOT.	
	(RANK, Original magnification ×40)	60
Figure 43	Photomicrograph showing a parakeratinized KCOT	
	with intact epithelial-CT interface, lumenal nest, and	
	inflammatory infiltrates. (RANK, Original	
	magnification ×40)	61
Figure 44	Photomicrograph showing a moderate cytoplasmic	
	immunopositivity for RANK in a parakeratinized	
	KCOT. (Original magnification ×40)	62

No.	Legend	Page
Figure 45	Higher magnification showing a moderate immunopositivity for RANK parakeratinized KCOT with signet ring cells, luminal keratin, corrugated thick keratin layer and impressive subepithelial hyalinization. (RANK, Original magnification ×40)	62
Figure 46	Scatter plot showing correlation between CD44 and	66
Figure 47	RANK in AM. Scatter plot showing correlation between CD44 and RANK in KCOT.	66

List of Tables

Table No.	Description	Page
Table 1	Categorization of the cases.	28
Table 2	Expression of CD44 in AM.	36
Table 3	Expression of RANK in AM.	38
Table 4	Expression of CD44 in KCOT.	39
Table 5	Expression of RANK in KCOT.	39
Table 6	Results of post-hoc Tukey HSD test.	63
Table 7	Results of post-hoc Tukey HSD test.	64
Table 8	Findings of MAF values of CD44 and RANK in AM and KCOT	65

INTRODUCTION

Most of odontogenic tumors occur intraosseously within the maxillofacial skeleton, while extra-osseous odontogenic tumors occur nearly always in the tooth-bearing mucosa. The clinical features of most benign odontogenic tumors are non-specific; benign odontogenic tumors show slow expansive growth with no or slight pain. In contrast, pain is the first and most common symptom followed by rapidly developing swelling in nearly all malignant odontogenic tumors. The tumor may erode or break through the gnathic bony cortex specially in pediatric populations [1].

Ameloblastoma (AM) is a very common odontogenic tumor in the oral cavity whose idiopathic nature manipulates scholars and clinicians. The etiopathogenesis of AM is controversial. The involved cellular changes - including proliferation, differentiation, senescence, tumorigenesis, etc - which are identified through the immuno-histochemical workup contribute significantly to our contemporary nosology of this aggressive benign tumor ^[2].

Ameloblastoma usually affects young adults in the fourth and fifth decades of life, causing local discomfort. It is characterized by a benign clinical behavior, possibly leading to local recurrences but distant metastases from AM are exceedingly rare. The involved cellular changes contribute significantly to our contemporary nosology of this aggressive benign tumor [3].

Keratocystic odontogenic tumor (KCOT), or odontogenic keratocyst, generally originates from the remnant of dental lamina or from basal cells of the oral epithelium. It predominantly develops in the mandible or maxilla, and occasionally on the gingiva as a peripheral type of manifestation ^[4]. Either a true cyst or a neoplasm, the scope of this study

does not include the controversial nature of KCOT, either neoplastic or cystic, because it concerns, more importantly, exploring the lesional destructive nature in terms of impressive osteooclastogenesis.

Both ameloblastoma and KCOT are characterized by a benign but locally invasive behavior, with a high risk of recurrence. Both can show an involvement of adjacent soft tissues, infiltration into the cancellous bone, and destructive growth. Read this way, hypothetically implicating, this study postulates that a plausible complex of CD44v6-OPN-RANK may exist, which is ushered toward exacerbating the osteoclastogenesis of ameloblastoma and KCOT ^[3,4].