



UNIVERSITI PUTRA MALAYSIA

CORRELATION BETWEEN HPV INFECTION AND HUMAN BETA-DEFENSIN mRNA EXPRESSION IN PTERYGIUM AND NORMAL CONJUNCTIVA EPITHELIAL CELLS

ABUBAKAR SAADATU ALIYU

FPSK(M) 2018 3



**CORRELATION BETWEEN HPV INFECTION AND HUMAN BETA-
DEFENSIN mRNA EXPRESSION IN PTERYGIUM AND NORMAL
CONJUNCTIVA EPITHELIAL CELLS**

By

ABUBAKAR SAADATU ALIYU

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirements for the Degree of Master of Science**

November 2017

COPYRIGHT

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia



DEDICATION

I would like to dedicate this thesis to my parents who have supported me with prayers since before I was born. Also, this thesis is dedicated to my beloved son and the entire humanity who believe in knowledge.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

CORRELATION BETWEEN HPV INFECTION AND HUMAN BETA-DEFENSIN mRNA EXPRESSION IN PTERYGIUM AND NORMAL CONJUNCTIVA EPITHELIAL CELLS

By

ABUBAKAR SAADATU ALIYU

November 2017

Chairman : Associate Professor Muhammad Hj Mohd Isa,
Faculty MD : Medicine and Health Science

Pterygium is a wing-shaped fibro-vascular proliferative lesion that originates from the bulbar conjunctiva and spreads to the corneal limbus and beyond. They are often seen on the nasal side horizontally in the interpalpebral fissure, but may appear on temporal sides too. It is known to cause irregular astigmatism which can compromise vision, corneal scarring, and restriction of ocular motility or ocular surface inflammation. Human β -defensins (HBDs) and viral oncogene such as human papillomavirus (HPV) may be involved in its etiology and development which are still a matter of contention. The aim of this study is to establish the relationship between HPV infection and human β -defensins mRNA gene expression in pterygium. Thirty pterygium and 30 normal conjunctiva epithelial samples were used in the study. Genomic DNA extracted from pterygium and normal conjunctiva was used to determine HPV-DNA using consensus general primer (GP5+/6+) by real time-PCR assay. Subsequently, HPV specific type 16 and 18 primers were used in HPV infected samples for subtyping. Human β -defensins DEFB1, DEFB4A, and DEFB109 mRNA expression were evaluated using real-time PCR and $\Delta\Delta$ -Ct method was used to calculate relative quantification of human beta defensins (HBDs) in Pterygium and normal conjunctiva epithelial cells. The relationship between HPV infection and human beta- defensins was analysed by Spearman's non parametric correlations for each bivariate pair. HPV viral DNA was detected in 29 of 30 Pterygium and 10 of 30 normal conjunctiva samples respectively, HPV 18 was the most common viral type identified. The expression of human β -defnsins DEFB1 and DEFB4A were up-regulated in pterygium when compared with that of normal conjunctiva of the same patient, while no significant difference was observed in DEFB4A expression. However, the expression of DEFB109 was significantly down- regulated in pterygium compared to that of normal conjunctiva.

Finally, there was positive correlation in DEFB1 and DEFB4A expression with HPV infection in normal conjunctiva epithelial cells. The current data suggest that HPV and HBDs may play a crucial role in pterygial development thus, may be considered as novel molecular target in understanding pterygium pathogenesis.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KORELASI ANTARA KADAR JANGKITAN HPV DAN EKSPRESI HUMAN BETA-DEFENSIN mRNA DI DALAM PTERYGIUM DAN SEL EPITHELIAL KONJUNKTIVA YANG NORMAL

Oleh

ABUBAKAR SAADATU ALIYU

November 2017

Pengerusi : Profesor Madya Hj Muhammad Hj Mohd Isa, MD

Fakulti : Perubatan dan Sains Kesihatan

Pterigium merupakan lesi proliferaatif vaskular fiber berbentuk sayap yang berpunca dari konjunktiva bulbus dan merebak ke limbus kornea dan sebaliknya. Lesi ini juga sering dilihat di bahagian nasal selari dengan interpalpebral fisur, atau di bahagian nasal dan temporal, ia boleh menyebabkan astigmatisme tidak tetap yang boleh menjejaskan penglihatan, kornea berparut, kekangan pergerakan okular atau radang permukaan okular. Human beta-defensins (HBDs) dan virus onkogen seperti HPV mungkin bertindak sebagai etiologi yang masih belum diketahui. Tujuan penyelidikan ini adalah untuk mengkaji korelasi antara kadar jangkitan HPV dengan ekspresi gen mRNA HBDs dalam pterigium. Sebanyak 30 sampel pterigium dan 30 sampel epitelial konjunktiva normal telah digunakan dalam kajian ini. Genomik DNA yang diekstrak daripada sampel-sampel tersebut digunakan dalam pengesanan DNA HPV menggunakan RT-qPCR. Primer umum (GP5+/6+) digunakan untuk mengesan HPV, kemudian primer jenis spesifik HPV 16 dan 18 digunakan untuk menentukan subtyping bagi sampel HPV-positif. Ekspresi mRNA HBDs DEFB1, DEFB4A dan DEFB109 telah dinilai menggunakan RT-qPCR and kaedah $\Delta\Delta$ -Ct digunakan untuk mengira pengkuantitian relatif ekspresi gen HBDs dalam pterigium dan sel epitelial konjunktiva normal. Hubungan antara kadar jangkitan HPV dan ekspresi HBDs telah diuji menggunakan analisis. Genom HPV dikesan dalam 29 sampel pterigium dan 10 sampel konjunktiva normal, HPV 18 merupakan serotaip virus yang paling banyak dikesan. Ekspresi HBDs DEFB1 dan DEFB4A adalah tinggi dalam pterigium apabila dibandingkan dengan sel epitelial konjunktiva normal dari pesakit yang sama walaupun ekspresi DEFB4A tidak menunjukkan perbezaan yang ketara secara statistik. Manakala ekspresi DEFB109 adalah rendah dalam secara ketara dalam pterigium berbanding dengan konjunktiva normal.

Secara keseluruhannya, kajian ini menunjukkan korelasi positif antara ekspresi DEB1 dan DEFB4A dengan jangkitan HPV dalam sel epithelial konjunktiva normal. Kajian ini juga menunjukkan bahawa HPV dan HBDS mungkin berperanan penting dalam patogenesis pterigium, dan boleh dijadikan target molekular dalam kajian lanjutan mengenai pterigium.



ACKNOWLEDGEMENTS

All praises and thanks are to Allah, Who has given me the ability to conduct this work. I would like to express the deepest gratitude to my supervisory committee chairman Associate Prof. Dr. Hj Muhammad bin Hj Mohd Isa for his invaluable assistance, support and guidance. He has been like a father to me throughout my study in Universiti Putra Malaysia. I would also like to sincerely thank Dr. Tan Sheu We for her direct and constant supervision in the lab, advice and suggestion in my research works. My deepest appreciation also goes to Dr. Nazri Omar for his advice, comment and suggestion, without whose effort this thesis would not have been possible.

I would like to take this opportunity to thank FUNDAMENTAL RESEARCH GRANT SCHEME (FRGS) for providing the research grant and Associate Prof. Dr. Hj Muhammad bin Hj Mohd Isa for providing me with graduate research assistance-ship under (FRGS).

Sincere thanks to Staff Nurse Halijah Binti Ahmad, Ophthalmology Department Hospital Serdang, Ophthalmology Unit Universiti Putra Malaysia and Laboratory of Vaccine and Immunotherapeutic Institute of Bioscience Universiti Putra Malaysia for their kindness and support during my study.

Finally, special thanks to all my family and friends especially my parents, my husband, my in-laws and Aunty Aisha Malah for their understanding and endless love throughout the period of my study.

I certify that a Thesis Examination Committee has met on 10 November 2017 to conduct the final examination of Abubakar Saadatu Aliyu on her thesis entitled "Correlation between HPV Infection and Human Beta-Defensin mRNA Expression in Pterygium and Normal Conjunctiva Epithelial Cells" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Maizatun Atmadini binti Abdullah, PhD

Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Noorjahan Banu binti Mohammed Alitheen, PhD

Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Alan Ong Han Kiat, PhD

Associate Professor
Universiti Tunku Abdul Rahman
Malaysia
(External Examiner)



NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 29 January 2018

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Hj Muhammad bin Hj Mohd Isa, MD

Associate Professor Medical
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Nazri Omar, MD, PhD

Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Tan Sheau Wei, PhD

Research Officer
Institute of Bioscience
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice –Chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity was upheld as according to the Universiti Putra Malaysia (Graduate studies) Rules 2003 (Revision 2012-2013) and the University Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No: Abubakar Saadatu Aliyu, GS43052

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as slated in the Universiti Putra Malaysia (Graduate studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman
of Supervisory
Committee: _____

Signature: _____
Name of Member
of Supervisory
Committee: _____

Signature: _____
Name of Member
of Supervisory
Committee: _____

TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENTS	v
APPROVAL	vi
DECLARATION	viii
LIST OF TABLES	xii
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS	xv
CHAPTER	
1 INTRODUCTION	1
1.1 Research Background	1
1.2 Problem Statement	3
1.3 Research Questions	3
1.4 General Objective	3
1.4.1 Specific Objectives	4
1.5 Research Hypotheses	4
2 LITERATURE REVIEW	5
2.1 The Human Eye	5
2.2 Conjunctiva	6
2.3 Degenerative Conditions of the Conjunctiva	6
2.3.1 Pinguecula	6
2.4 Pterygium	6
2.4.1 Ultraviolet (UV) Radiation and Pterygium	8
2.4.2 Pathophysiology of pterygium	9
2.4.2.1 Inflammation	9
2.4.2.2 Cell proliferation and migration	9
2.4.2.3 Angiogenesis	10
2.4.2.4 Extracellular matrix remodelling	10
2.4.3 Genetic Factors, Viral Infections and Pterygium	10
2.5 Papillomaviruse	11
2.5.1 Human Papillomaviruses	12
2.5.2 Life Cycle of High Risk HPV	14
2.5.3 HPV Genomic Organisation	17
2.6 Defensins	17
2.6.1 Human (β) Defensins	18
2.6.2 Anti-Viral Activity of Defensins	19
2.6.3 Role of Defensins in Inflammation and Disease	20

3	MATERIALS AND METHODS	23
3.1	Ethics Approval	23
3.2	Experimental Design	23
	3.2.1 Inclusion Criteria	23
	3.2.2 Exclusion Criteria	23
3.3	Collection of Samples from Patients	24
3.4	Sample Processing	25
	The pterygium tissues	25
3.5	HPV DNA Detection	25
	3.5.1 DNA Extraction	25
	3.5.2 Human β -globin Gene as Internal Control in qPCR Amplification	25
3.6	Detection of HPV DNA Using Real-Time PCR	26
3.7	Typing of HPV Using HPV 16 and HPV 18 Primers	26
3.8	Human β -Defensin mRNA Expression	27
3.9	RNA Extraction	27
	3.9.1 cDNA Synthesis	27
3.10	Real-Time PCR	27
4	RESULTS	29
4.1	Patient Demographics	29
4.2	HPV Detection and Typing using L1 region by Real Time-PCR	30
4.3	Expression of Human β -Defensins (DEFB1, DEFB4A and DEFB109) in Pterygium and Normal Conjunctiva Samples	37
	4.3.1 RNA Quality Assessment	37
4.4	Gene Expression Analysis	41
4.5	Correlation between HPV Infection and Human β -defensins mRNA Expression in Pterygium and Normal Conjunctiva	55
5	DISCUSSION	57
5.1	HPV Detection and Typing in Pterygium and Normal Conjunctiva	57
5.2	Expression of Human β -Defensins gene in Pterygium	60
5.3	Correlation between HPV Infection and Human β -defensins mRNA Expression in Pterygium and Normal Conjunctiva	62
6	CONCLUSION	65
6.1	Recomendation	66
	REFERENCES	67
	APPENDICES	90
	BIODATA OF STUDENT	99
	LIST OF PUBLICATIONS	100

LIST OF TABLES

Table	Page
2.1 Characteristics of Defensins	18
3.1 List of Primers for HPV Detection and Typing	26
3.2 List of Human β -Defensins Primers	28
4.1 Demographics of the Patient	29
4.2 Patient Demographics and HPV Types	37
4.3 Pattern of Expression in Samples that both Normal and Pterygium are infected with HPV	56

LIST OF FIGURES

Figure	Page	
2.1	Anatomy of the eye	5
2.2	Image of inflammatory nasal pterygium before surgery	7
2.3	HPV DNA	11
2.4	Phylogeny of 189 PV types base on L1 nucleotides sequence	12
2.5	HPV genome	13
2.6	Normal life cycle of HPV	15
2.7	Genomic organisation of HPV 16	16
2.8	Antiviral mechanism of defensins	20
3.1	Experimental design	24
4.1	Recurrence rate of pterygium with age	30
4.2	Amplification and melting curve of plot β -globin	31
4.3	Amplification and melting curve plot of GP5+/6+ in pterygium	32
4.4	Amplification and melting curve plot of GP5+/6+ in normal Conjunctiva	33
4.5	Amplification and melting curve plot of HPV 16 and 18 in pterygium	35
4.6	Amplification and melting curve plot of HPV 16 and 18 in normal conjunctiva	36
4.7	Standard curve for DEFB109	39
4.8	Standard curve for DEFB1	40
4.9	Standard curve for DEFB4A	41
4.10	T1-30 expression of DEFB1, DEFB4A and DEFB109 in normal conjunctiva and pterygium	46
4.11	Average expression of DEFB1 N=30	54

4.12	Average expression of DEFB4A N=23	54
4.13	Average expression of DEFB109 N=30	55



LIST OF ABBREVIATIONS

ADP	Adenosine 5'-diphosphate
AD	Transactivation domain
APC	Antigen Presenting Cell
AP1	Activator Protein 1
BD	Bond distance
Bp	Base pair
BP	Base peak
C	Carbon
CIN	Chromosomal instability
CBP	CREB-binding protein
CD4+	
CREB	Cyclic AMP response element-binding protein
COX-2	Cyclooxygenase 2
DHA	Hydrogen donor; hydrogen; hydrogen acceptor
D	Day
DC	Dendritic cells
Dntp	Deoxynucleotide triphosphates
DBD	DNA binding domain
DNA	Deoxyribonucleic acid
EGFR	Epidermal growth factor receptor
ECM	Extracellular matrix
EBV	Epstein-Barr Virus
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor

ERK	Extracellular signal-regulated kinase
FPRL1	Formyl peptide receptor-like 1
ERK	Extracellular signal-regulated kinase
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
Bfgf	Growth factors
H ₂ O	Water
HB	Hydrogen bond
HBD	Human β -defensin
HB-EGF	Heparin-binding epidermal growth factor
HCAP18	Human cationic anti-microbial protein 18
HD	Human defensin (epithelial-derived human α -defensin)
HIF-1 α	Hypoxia inducible factor 1 α
HIV	Human Immunodeficiency Virus
HPV	Human Papilloma Virus
HNP	Human neutrophil peptide (neutrophil-derived human α -
HBDs	Human beta-defensins
HDM2	Human double minute 2
HPV	Human papillomavirus
Hogg1	Human 8-oxoguanine DNA glycosylase 1
HSV	Herpes simplex virus
IARC	International agency for cancer research
IU	International unit
IL-1 β	Interleukin 1 β 16
IFN- γ	Interferon γ
I κ B	Inhibitor of K κ b
PS3	Integrin alpha-

ICTV	International Committee on the Taxonomy of Viruses
L	Litre
LOH	Loss of heterozygosity
LTA	Lipoteichoic acid
LPS	Lipopolysaccharide
MAGUK	Membrane associated guanylate kinase
mg/ml	Milligrams per millilitre
Min	Minute
MMP7	Metalloproteinase-7
m.p	Melting point
Mg	Milligrams
MDM2	Murine double minute 2
MAPK	Mitogen-activated protein kinase
MMC	Mitomycin C
MMPs	Matrix metalloproteinases
SMA	Spinalmuscular atrophy
SMN	Survival motor neuron
Mrna	Messenger RNA
NDP	Nucleoside diphosphates
NF-Kb	Nuclear Factor kappa B
NLS	Nuclear localisation signal
NF-Kb	Nuclear factor Kb
NK cells	Natural killer cells
O	Oxygen
ODC	Ornithine decarboxylase
OSCC	Oral squamous cell carcinoma

PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PMA	Phorbol 12-myristate 13-acetate
p53	Tumour suppressor
RNA	Ribonucleic acid
RP	Ribosomal proteins
Rpm	Revolutions per minute
RT-PCR	Real- time-polymerase chain reaction
S	Second
SCCHN	Squamous cell carcinoma of the head and neck
SCID	Severe combined immunodeficiency
STAT	Signal transducer and activator of transcription
TAM	Tumour associated macrophage
TGF α	Transforming growth factor α : Ligand to EGFR receptor
TNF α	Tumor necrosis factor α
TNM:	A clinical staging system for grading SCCHN tumours: (T: refers to tumour size at the primary tumour site, N: refers to the status of cervical lymph nodes, and M: refers to the presence or absence of distant metastases)
TBP	TATA binding protein
TIL	Tumor infiltrating lymphocytes
TLR	Toll-like receptor
T	Triplets
UVR	Ultraviolet radiation
Vwf	von Willebrand factor
VLP	virus-like particles
WHO	World Health Organisation

%	Percent
°C	Degree Celsius
µg/ml	Microgram per millilitre
µM	Micro molar
µL	Micro litre
>	Greater than
Α	Alpha
β	Beta



© COPYRIGHT UPM

CHAPTER 1

INTRODUCTION

1.1 Research Background

Pterygium is a wing-shaped fibro vascular proliferative lesion that originates from the bulbar conjunctiva, which spreads to the corneal limbus and beyond. They are often seen on the nasal conjunctiva in the interpalpebral fissure although they can appear either on or on both the nasal and temporal sides (Masters and Harris 2015). The prevalence rate of pterygium as reported in different studies varies widely with age, gender and geographical location. They are mostly observed in people from tropical climates, but pterygium can be found in over 200 million people worldwide (Lucas et al. 2008). The exact aetiology of pterygium is not fully understood. Previous studies suggested that pterygium was highly associated with ultraviolet radiation (UVR) exposure. Molecular alterations associated with pterygium include loss of heterozygosity (LOH), point mutations of proto-oncogenes (K-ras) and alterations in the expression of tumour suppressor genes (p53 or p63) and nuclear factor (cyclic AMP response element-binding protein CREB) (Nubile et al. 2013). Other findings in pterygium include the frequent detection of HPV DNA, over expression of various ocular surface proteins, including defensins and phospholipases D, as well as the up-regulation of growth factors, such as bFGF or VEGF (Detorakis and Spandidos 2009).

Pterygium can cause irregular corneal astigmatism, corneal scarring and restriction of ocular motility. In some severe cases, pterygium may result in visual impairment if it approaches visual axis or chronic ocular surface inflammation (Liu et al. 2013; Julio et al. 2013). Traditionally, pterygium is regarded as a disease of the elderly, leading to suspicion of primary degenerative cause. Pterygium management usually depends on the size and extent of the pterygium. A small pterygium can be treated with mild steroid eye drops (Rachmiel, Leiba, and Levartovsky 1995) while a large size would require surgery (Bozkir, Yilmaz, and Maden 2008; Ozkurt et al. 2009; Varssano et al. 2013) which is normally enhanced by the use of antimetabolites. Recent progress in the biochemical and molecular pathogenesis of pterygium in recent years has helped in the use of minimally invasive methods of treatment like minimally invasive pterygium excision MIPE (Bozkir, Yilmaz, and Maden 2008).

The pathological mechanism that causes pterygium appears to be multi factorial as the exact mechanism has yet to be elucidated (Chalkia, Spandidos, and Detorakis 2015). Several theories have been put forward to clarify how pterygium progresses include; autosomal dominant mode of inheritance, immunologically-mediated, tear film disruptions, chronic UVR exposure, and viral infection (Di Girolamo 2012).

Human papillomavirus (HPV) are small double-stranded circular DNA non-enveloped viruses with genome size of approximately 8000 bp, encoding early-region sequences or open-reading frames (ORF) designated as E1–E8 that vary in number between HPV types and are expressed shortly after HPV infection. The viral genome also contains two latent regions labeled L1 and L2, which encode the capsid protein that has a role in viral entry. There are over 200 different types of HPV identified but not all have been characterized as oncogenic virus (Di Girolamo 2012). HPV viruses can infect stratified squamous mucosal, cutaneous, and other epithelia cells of mammals. They are found to be associated with squamo-proliferative lesions of the anogenital region, skin and oropharynx. Several studies have shown HPV involvement in the pathogenesis of conjunctival neoplastic lesions, including papilloma and squamous cell carcinoma (Chalkia, Spandidos, and Detorakis 2015). The involvement of HPV as a co-factor in the pathogenesis of pterygium, suggested by several studies using PCR and immunohistochemical techniques, remains controversial. Moreover, a marked variation in the prevalence of HPV in ophthalmic pterygium has been reported by several studies. The HPV infection rate was found to be high as 50-100% pterygium in some studies (Detorakis, Drakonaki, and Spandidos 2000; Di Girolamo 2012; Chalkia, Spandidos, and Detorakis 2013; Chalkia, Spandidos, and Detorakis 2015) but was unable to be detected in other studies (Schellini et al. 2006; Sjö et al. 2007; Hsiao et al. 2010; Masters and Harris 2015).

Defensin family consists of small (2-6 kDa) cationic antimicrobial peptides between 20-50 amino acids with six evolutionary conserved cysteine residues (Liu et al. 1997). They form disulphide bridges in three pairs, giving rise to three anti-parallel beta sheets structure that assume evolutionary conserved structural fold (Jia et al. 2001; Ganz 2003). Apart from the six cysteine residues, members of the defensin family have low sequence homology. This observation was believed to result in the difference of characters between all the family members. In humans, 6 α -defensins and 11 human β -defensins have been isolated (Zhou et al. 2013). Human β -defensins (HBDs) are produced by epithelial cells lining the body surface and acts as natural antibiotics and immunoregulators thus, providing the first line of defence against infection, inflammation and wound healing (Zhou et al. 2013). HBDs have wide-spectrum of antimicrobial and biological activities with little risk of developing resistances. They can also inhibit many steps in viral infection as well as growth of microbes (Wilson, Wiens, and Smith 2013). The expression of human β -defensins is either constitutive or inducible in response to infection or tissue injury (Dhople, Krukemeyer, and Ramamoorthy 2006; Wehkamp et al. 2007). When induced they normally result in their most effective site-specific response.

Human β -defensins (hBDs) demonstrate proinflammatory activity by binding to certain receptors. For example, human β -defensin 2 (hBD-2) and human β -defensin 1 (hBD1) bind to chemokine receptor 6 (CCR6) leading to increment in chemoattraction of both CD4⁺ memory T-helper cells and immature dendritic cells (Yang et al. 1999). hBDs can also play a role in carcinogenesis of epithelial tumours. Changes in expression of human β -defensins was observed in epithelial-derived cancers such as prostatic cancer, basal cell carcinoma, oral squamous cell carcinoma (OSCC) and renal cell carcinoma (Al-Rayahi and Sanyi 2015). The variation in expression pattern

of β -defensins in such cancers has led researchers to investigate anti-tumour potential of β -defensins.

1.2 Problem Statement

The pathogenesis of pterygium is not fully understood and several factors have been implicated in the formation of pterygium. Central to these factors are the ultra violet ray (UVR) damage while the role of oncogenic viral infection is controversial. The presence of Human papillomavirus (HPV) in patients diagnosed with pterygium was found to be as high as 50-100% in several studies but failed to be detected in other studies. Insertion of HPV 16/18 DNA into the host chromosome to express the E6/E7 oncoprotein plays crucial roles in HPV-induced cervical carcinogenesis. Human β -defensin is produced by the epithelial cells lining the body surface, thus providing immediate and direct response towards external challenges. Defensins play important role in the innate immunity and adaptive immunity. They protect the host by conferring first line protection against invading organisms such as bacteria, fungi and viruses. In addition to the antimicrobial role, defensins are involved in various cellular processes such as chemo-attraction, anti-cancer function wound healing and cell proliferation. Based on these functions of the defensins and given the events underlying the pathogenesis of pterygium, HPV and human β -defensins may influence the pathogenesis of pterygium. However, the relationship between them has not been established and become the focus of this study.

1.3 Research Questions

1. Is the HPV infection significantly increased in pterygium than in normal conjunctiva?
2. Is the human beta-defensins genes up- regulated or down-regulated in pterygium compared to that of normal conjunctiva?
3. What is the correlation between the human beta defensins gene and HPV infection rate in pterygium?

1.4 General Objective

The aim of this study was to determine the correlation between Human Papillomavirus (HPV) infection and Human β -defensin mRNA gene expression using real time Polymerase Chain Reaction (qPCR) in pterygium and normal conjunctival epithelial cells from patients undergoing the pterygium excision.

1.4.1 Specific Objectives

1. To determine the prevalence of human papillomavirus infection in pterygium and normal conjunctiva epithelial samples.
2. To determine high-risk human papillomavirus HPV-16 and HPV-18 in HPV infected pterygium and normal conjunctiva samples.
3. To determine DEFB1, DEFB4A, and DEFB109 mRNA expression in pterygium and normal conjunctiva epithelial samples.
4. To determine the correlation between the HPV infection and the human β -defensins gene expression in pterygium and normal conjunctiva samples.

1.5 Research Hypotheses

- i. The HPV infection will increase in pterygium compared to normal conjunctiva samples.
- ii. The human β -defensins gene expression will reduce in pterygium compared to normal conjunctiva samples.
- iii. Down regulation of human β -defensin mRNA expression would increase the risk of HPV infection in pterygium.

REFERENCES

- Abedin, Asiya, Imran Mohammed, Andrew Hopkinson, and Harminder S. Dua. 2008. "A Novel Antimicrobial Peptide on the Ocular Surface Shows Decreased Expression in Inflammation and Infection." *Investigative Ophthalmology & Visual Science* 49 (1): 28–33.
- Al-Rayahi, Izzat AM, and Raghad HH Sanyi. 2015. "The Overlapping Roles of Antimicrobial Peptides and Complement in Recruitment and Activation of Tumor-Associated Inflammatory Cells." *Frontiers in Immunology* 6 (1): 1–5.
- Albrethsen, Jakob, Christian H. Møller, Jesper Olsen, Hans Raskov, and Steen Gammeltoft. 2006. "Human Neutrophil Peptides 1, 2 and 3 Are Biochemical Markers for Metastatic Colorectal Cancer." *European Journal of Cancer* 42 (17): 3057–3064.
- Alp, S., M. Skrygan, R. Schlottmann, A. Kreuter, J. M. Otte, W. E. Schmidt, N. H. Brockmeyer, and A. Bastian. 2005. "Expression of Beta-Defensin 1 and 2 in Nasal Epithelial Cells and Alveolar Macrophages from HIV-Infected Patients." *European Journal of Medical Research* 10 (1): 1–6.
- Alpay, Atilla, Suat Hayri Uğurbaş, and Berktuğ Erdoğan. 2009. "Comparing Techniques for Pterygium Surgery." *Clinical Ophthalmology* 3 (1): 69–74.
- Aminlari, Ardalan, Ravi Singh, and David Liang. 2010. "Management of Pterygium." *Eyenet* :37–38.
- Andresen, Ellen, Gunar Günther, Jörn Bullwinkel, Christoph Lange, and Holger Heine. 2011. "Increased Expression of Beta-Defensin 1 (DEFB1) in Chronic Obstructive Pulmonary Disease." *PLoS ONE* 6 (7): e21898.
- Ang, Marcus, Xiang Li, Wanling Wong, Yingfeng Zheng, Daniel Chua, Ainur Rahman, Seang Mei Saw, Donald T H Tan, and Tien Yin Wong. 2012. "Prevalence of and Racial Differences in Pterygium: A Multiethnic Population Study in Asians." *Ophthalmology* 119 (8): 1509–1515.
- Anguria, Peter, James Kitinya, Sam Ntuli, and Trevor Carmichael. 2014. "The Role of Heredity in Pterygium Development." *International Journal of Ophthalmology* 7 (3): 563–573.
- Apt, Doris, Robin M. Watts, Guntram Suske, and Hans-Ulrich Bernard. 1996. "High Sp1/Sp3 Ratios in Epithelial Cells during Epithelial Differentiation and Cellular Transformation Correlate with the Activation of the HPV-16 Promoter." *Virology* 224 (1): 281–291.

- Aspiotis, M., E. Tsanou, S. Gorezis, E. Ioachim, A. Skyrilas, M. Stefanidou, and V. Malamou-Mitsi. 2007. "Angiogenesis in Pterygium: Study of Microvessel Density, Vascular Endothelial Growth Factor, and Thrombospondin-1." *Eye* 21 (8): 1095–1101.
- Befus, A. Dean, Connie Mowat, Mark Gilchrist, Jing Hu, Samuel Solomon, and Andrew Bateman. 1999. "Neutrophil Defensins Induce Histamine Secretion from Mast Cells: Mechanisms of Action." *Journal of Immunology* 163 (2): 947–953.
- Bernard, Hans-Ulrich, Robert D. Burk, Zigui Chen, Koenraad Van Doorslaer, and Ethel-michele de Villiers. 2010. "Classification of Papillomaviruses PVs Based on 189 PV Types and Proposal of Taxonomic Amendments." *Virology* 401 (1): 70–79.
- Bernard, Hans-Ulrich. 2013. "Taxonomy and Phylogeny of Papillomaviruses: An Overview and Recent Developments." *Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases* 18 (8): 357–361.
- Biryukov, Jennifer, and Craig Meyers. 2015. "Papillomavirus Infectious Pathways: A Comparison of Systems." *Viruses* 7 (8): 4303–4325.
- Bolaños-jiménez, Rodrigo, Alejandro Navas, Erika Paulina López-lizárraga, Francesc March de Ribot, Alexandra Peña, Enrique O. Graue-hernández, and Yonathan Garfias. 2015. "Ocular Surface as Barrier of Innate Immunity." *Open Ophthalmology Journal* 9: 49–55.
- Bonello, Karl, and Renald Blundell. 2016. "The Role of the Human Papillomavirus (HPV) in Cervical Cancer: A Review about HPV-Induced Carcinogenesis and Its Epidemiology, Diagnosis, Management and Prevention." *Journal of Medical Students* 4 (1): 26–32.
- Boughan, Parjeet K, Richard H Argent, Mathilde Body-Malapel, Jong-Hwan Park, Katie E Ewings, Andrew G Bowie, Shao Jin Ong, et al. 2006. "Nucleotide-Binding Oligomerization Domain-1 and Epidermal Growth Factor Receptor: Critical Regulators of Beta-Defensins during Helicobacter Pylori Infection." *Journal of Biological Chemistry* 281 (17): 11637–11648.
- Bozkir, N., S. Yilmaz, and A. Maden. 2008. "Minimally Invasive Pterygium Surgery: A New Approach for Prevention of Recurrence." *European Journal of Ophthalmology* 18 (1): 27–31.
- Braaten, Kari P., and Marc R. Laufer. 2008. "Human Papillomavirus (HPV), HPV-Related Disease, and the HPV Vaccine." *Reviews in Obstetrics & Gynecology* 1 (1): 2–10.

- Buitrago-Pérez, Águeda, Mariam Hachimi, Marta Dueñas, Belén Lloveras, Almudena Santos, Almudena Holguín, Blanca Duarte, et al. 2012. "A Humanized Mouse Model of HPV-Associated Pathology Driven by E7 Expression." *PLoS ONE* 7 (7): 1–18.
- Bullard, Rebecca S., Willietta Gibson, Sudeep K. Bose, Jamila K. Belgrave, Andre C. Eaddy, Corey J. Wright, Debra J. Hazen-Martin, et al. 2008. "Functional Analysis of the Host Defense Peptide Human Beta Defensin-1: New Insight into Its Potential Role in Cancer." *Molecular Immunology* 45 (3): 839–848.
- Burd, Eileen M. 2003. "Human Papillomavirus and Cervical Cancer." *Clinical Microbiology Reviews* 16 (1): 1–17.
- Cameron, Malcolm E. 1983. "Histology of pterygium: an electron microscopic study." *British Journal of Ophthalmology* 67 (9): 604-608.
- Chalkia, Aikaterini K., Demetrios A. Spandidos, and Efstathios T. Detorakis. 2013. "Viral Involvement in the Pathogenesis and Clinical Features of Ophthalmic Pterygium (Review)." *International Journal of Molecular Medicine* 32 (3): 539-543.
- Chaly, Yu V., E. M. Paleolog, T. S. Kolesnikova, I. I. Tikhonov, E. V. Petratchenko, and N. N. Voitenok. 2000. "Neutrophil Alpha-Defensin Human Neutrophil Peptide Modulates Cytokine Production in Human Monocytes and Adhesion Molecule Expression in Endothelial Cells." *European Cytokine Network* 11 (2): 257–266.
- Chang, Theresa L., Jesus Jr Vargas, Armando DelPortillo, and Mary E. Klotman. 2005. "Dual Role of Alpha-Defensin-1 in Anti-HIV-1 Innate Immunity." *The Journal of Clinical Investigation* 115 (3): 765–73.
- Chen, Jan-Kan, Ray J-F. Tsai, and Song-Shu Lin. 1994 "Fibroblasts isolated from human pterygia exhibit transformed cell characteristics." *In Vitro Cellular & Developmental Biology-Animal* 30 (4): 243-248.
- Chen, Q. X., C. Lv, L. X. Huang, B. L. Cheng, G. H. Xie, S. J. Wu, and X. M. Fang. 2007. "Genomic Variations within DEFB1 Are Associated with the Susceptibility to and the Fatal Outcome of Severe Sepsis in Chinese Han Population." *Genes and Immunity* 8 (5): 439–443.
- Chiang, Chun-Chi, Yi-Yu Tsai, Da-Tian Bau, Ya-Wen Cheng, Sung-Huei Tseng, Rou-Fen Wang, and Fuu-Jen Tsai. 2010. "Pterygium and Genetic Polymorphisms of the DNA Repair Enzymes XRCC1, XPA, and XPD." *Molecular Vision* 16 (11): 698–704.

- Chong, Kong T., Liangbin Xiang, Xiaohong Wang, Eunjoo L. Jun, Long-fu Xi, and John M. Schweinfurth. 2006. "High level expression of human epithelial β -defensins (hBD-1, 2 and 3) in in Papillomavirus Induced Lesions." *Virology Journal* 3 (1): 75.
- Chong, Pei Pei, Chee Hong Tung, Nurul Asyikin, Misako Yajima, Fee Wai, Crystale Lim, Siew Yeng, Eng Soon Go, Cordelia M L Chan, and Nobuyo Yawata. 2014. "Prevalence and Viral Load of Oncogenic Human Papillomavirus (HPV) in Pterygia in Multi-Ethnic Patients in the Malay Peninsula." *Acta Ophthalmologica* 92 (7): 569–579.
- Chui, Jeanie, Nick Di Girolamo, Denis Wakefield, and Minas T. Coroneo. 2008. "The Pathogenesis of Pterygium: Current Concepts and Their Therapeutic Implications." *The Ocular Surface* 6 (1): 24–43.
- Chui, Jeanie, Minas T. Coroneo, Lien T. Tat, Roger Crouch, Denis Wakefield, and Nick Di Girolamo. 2011. "Ophthalmic Pterygium: A Stem Cell Disorder with Premalignant Features." *American Journal of Pathology* 178 (2): 817–827.
- Conejo-Garcia, Jose R., Fabian Benencia, Maria-Cecilia Courreges, Eugene Kang, Alisha Mohamed-Hadley, Ronald J. Buckanovich, David O. Holtz, et al. 2004. "Tumor-Infiltrating Dendritic Cell Precursors Recruited by a Beta-Defensin Contribute to Vasculogenesis under the Influence of VEGF-A." *Nature Medicine* 10 (9): 950–958.
- Conway, M. J., and C. Meyers. 2009. "Replication and Assembly of Human Papillomaviruses." *Journal of Dental Research* 88 (4): 307–317.
- Conway, Michael J., Samina Alam, Neil D. Christensen, and Craig Meyers. 2010. "Overlapping and Independent Structural Roles for Human Papillomavirus Type 16 L2 Conserved Cysteines." *Virology* 393 (2): 295–303.
- Corleis, Bjorn, William Gostic, and Douglas Kwon. 2013. "Protective Role of Anti-Viral Human Defensins during Acute HIV Infection (P4049)." *The Journal of Immunology* 190 (1 Supplement): 125-131.
- Cornet, Iris, Véronique Bouvard, Maria Saveria Campo, Miranda Thomas, Lawrence Banks, Lutz Gissmann, Jérôme Lamartine, Bakary S. Sylla, Rosita Accardi, and Massimo Tommasino. 2012. "Comparative Analysis of Transforming Properties of E6 and E7 from Different Beta Human Papillomavirus Types." *Journal of Virology* 86 (4): 2366–2370.
- Coroneo, M. T. 1993. "Pterygium as an Early Indicator of Ultraviolet Insolation: A Hypothesis." *The British Journal of Ophthalmology* 77 (11): 734–739.
- Coroneo, M. T., Nick Di Girolamo, and Denis Wakefield. 1999. "The Pathogenesis of Pterygia." *Current Opinion in Ophthalmology* 10 (4): 282–288.

- Coussens, Lisa M., and Zena Werb. 2010. "Inflammation and Cancer" *Nature*, 420 (6917): 860-867.
- Daher, Kathleen A., Michael E. Selsted, and Robert I. Lehrer. 1986. "Direct Inactivation of Viruses by Human Granulocyte Defensins." *Journal of Virology* 60 (3): 1068-1074.
- De Villiers, Ethel-Michele, Claude Fauquet, Thomas R. Broker, Hans-Ulrich Bernard, and Harald Zur Hausen. 2004. "Classification of Papillomaviruses." *Virology* 324 (1): 17-27.
- Detorakis, Efstathios T., E. E. Drakonaki, and Demetrios A. Spandidos. 2000. "Molecular Genetic Alterations and Viral Presence in Ophthalmic Pterygium." *International Journal of Molecular Medicine*.
- Detorakis, Efstathios T., George Sourvinos, and Demetrios A. Spandidos. 2001. "Detection of Herpes Simplex Virus and Human Papilloma Virus in Ophthalmic Pterygium." *Cornea* 20 (2): 164-167.
- Detorakis, Efstathios T., and Demetrios A. Spandidos. 2009. "Pathogenetic Mechanisms and Treatment Options for Ophthalmic Pterygium: Trends and Perspectives (Review)." *International Journal of Molecular Medicine* 23 (4): 439-447
- Detorakis, Efstathios T., George Kymionis, Michael Tsatsos, and Demetrios A. Spandidos. 2016. "Pterygium Concomitant with Other Ocular Surface Lesions: Clinical Implications and Pathogenetic Links." *Experimental and Therapeutic Medicine* 11 (1): 69-72
- Dhople, Vishnu, Amy Krukemeyer, and Ayyalusamy Ramamoorthy. 2006. "The Human Beta-Defensin-3, an Antibacterial Peptide with Multiple Biological Functions." *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1758 (9): 1499-1512.
- Di Girolamo, Nick, Rakesh K. Kumar, Minas T. Coroneo, and Denis Wakefield. 2002. "UVB-Mediated Induction of Interleukin-6 and -8 in Pterygia and Cultured Human Pterygium Epithelial Cells and Cultured Human Pterygium Epithelial Cells." *Investigative Ophthalmology & Visual Science* 43 (11): 3430-3437.
- Di Girolamo, Nick, Jeanie Chui, Minas T. Coroneo, and Denis Wakefield. 2004. "Pathogenesis of Pterygia: Role of Cytokines, Growth Factors, and Matrix Metalloproteinases." *Progress in Retinal and Eye Research* 23 (2): 195-228
- Di Girolamo, Nick, Minas Coroneo, and Denis Wakefield. 2005. "Epidermal Growth Factor Receptor Signaling Is Partially Responsible for the Increased Matrix Metalloproteinase-1 Expression in Ocular Epithelial Cells after UVB Radiation." *The American Journal of Pathology* 167 (2): 489-503.

- Di Girolamo, Nick, Denis Wakefield, and Minas T. Coroneo Nick Di, Denis Wakefield, and Minas T Coroneo. 2006. "UVB-Mediated Induction of Cytokines and Growth Factors in Pterygium Epithelial Cells Involves Cell Surface Receptors and Intracellular Signaling," *Investigative Ophthalmology & Visual Science* 47 (6): 2430-2437.
- Di Girolamo, Nick. 2012. "Association of Human Papilloma Virus with Pterygia and Ocular-Surface Squamous Neoplasia." *Eye* 26 (2): 202–211.
- Diakos, Connie I., Kellie A. Charles, Donald C. McMillan, and Stephen J. Clarke. 2014. "Cancer-Related Inflammation and Treatment Effectiveness," *The Lancet Oncology*, 15 (11): 493–503.
- Ding, Jian, Aprille Rapista, Natalia Teleshova, Goar Mosoyan, Gary A. Jarvis, Mary E. Klotman, and Theresa L. Chang. 2010. "Neisseria Gonorrhoeae Enhances HIV-1 Infection of Primary Resting CD4+ T Cells through TLR2 Activation." *Journal of Immunology* 184 (6): 2814–2824.
- Ding, Jian, Carley Tasker, Kimyata Valere, Tiina Sihvonen, Dante B. Descalzi-Montoya, Wuyuan Lu, and Theresa L. Chang. 2013. "Anti-HIV Activity of Human Defensin 5 in Primary CD4+ T Cells under Serum-Deprived Conditions Is a Consequence of Defensin-Mediated Cytotoxicity." *PLoS ONE* 8 (9): 1–11.
- Doorbar, John. 2006. "Molecular Biology of Human Papillomavirus Infection and Cervical Cancer." *Clinical Science* 110 (5): 525–541.
- Doorbar, John, Wim Quint, Lawrence Banks, Ignacio G. Bravo, Mark Stoler, Tom R. Broker, and Margaret A. Stanley. 2012. "The Biology and Life-Cycle of Human Papillomaviruses." *Vaccine* 30 (11): F55–F70.
- Doss, Mona, Mitchell R. White, Tesfaldet Tecele, and Kevan L. Hartshorn. 2010. "Human Defensins and LL-37 in Mucosal Immunity." *Journal of Leukocyte Biology* 87 (1): 79–92.
- Droin, Nathalie, Arnaud Jacquelin, Jean-Baptiste Hendra, Cindy Racœur, Caroline Truntzer, Delphine Pecqueur, Naima Benikhlef, Marion Ciudad, Leslie Guery, Valérie Jooste, Erick Dufour, Pierre Fenaux, Bruno Quesnel, Olivier Kosmider, Michaëla Fontenay, Patrick Ducoroy and Eric Solary. 2010. "Alpha-Defensins Secreted by Dysplastic Granulocytes Inhibit the Differentiation of Monocytes in Chronic Myelomonocytic Leukemia." *Blood* 115 (1): 78–88.
- Droutsas, K, and W Sekundo. 2010. "Epidemiologie Des Pterygiums." *Der Ophthalmologe* 107 (6): 511–516.

- Duits, Louise A, Bep Ravensbergen, Mirjam Rademaker, Pieter S Hiemstra, and Peter H Nibbering. 2002. "Expression of Beta-Defensin 1 and 2 mRNA by Human Monocytes, Macrophages and Dendritic Cells." *Immunology* 106 (4): 517–525.
- Dushku, Nicholas, Molykuty K. John, Gregory S. Schultz, and Ted W. Reid. 2001. "Pterygia Pathogenesis" *Archives of Ophthalmology* 119 (5): 695-706.
- Erhart, W., Ö Alkasi, G. Brunke, F. Wegener, N. Maass, N. Arnold, A. Arlt, and I. Meinhold-Heerlein. 2011. "Induction of Human β -Defensins and Psoriasin in Vulvovaginal Human Papillomavirus-Associated Lesions." *Journal of Infectious Diseases* 204 (3): 391–399.
- Fellermann, Klaus, Daniel E Stange, Elke Schaeffeler, Hartmut Schmalzl, Jan Wehkamp, Charles L. Bevins, Walter Reinisch, Alexander Teml, Matthias Schwab, Peter Lichter, Bernhard Radlwimmer and Eduard F. Stange. 2006. "A Chromosome 8 Gene-Cluster Polymorphism with Low Human Beta-Defensin 2 Gene Copy Number Predisposes to Crohn Disease of the Colon." *American Journal of Human Genetics* 79 (3): 439–448.
- Feng, Zhimin, George R. Dubyak, Michael M. Lederman, and Aaron Weinberg. 2006. "Cutting Edge: Human Beta Defensin 3—A Novel Antagonist of the HIV-1 Coreceptor CXCR4." *Journal of Immunology* 177 (2): 782–786.
- Frantz, Christian, Kathleen M. Stewart, Valerie M. Weaver, Christian Frantz, Kathleen M. Stewart, and Valerie M. Weaver. 2010. "The Extracellular Matrix at a Glance The Extracellular Matrix at a Glance" *Journal Cell Science* 123 (24): 4195-4200.
- Ganz, Tomas. 2003. "DEFENSINS: Antimicrobial peptides of innate immunity" *Nature Reviews Immunology* 3 (9): 710-720.
- Garreis, Fabian, Thomas Schlorf, Dieter Worlitzsch, Philipp Steven, Lars Bräuer, Kristin Jäger, and Friedrich P. Paulsen. 2010. "Roles of Human β -Defensins in Innate Immune Defense at the Ocular Surface: Arming and Alarming Corneal and Conjunctival Epithelial Cells." *Histochemistry and Cell Biology* 134 (1): 59–73.
- George, John T., Parjeet K. Boughan, Haris Karageorgiou, and Mona Bajaj-Elliott. 2003. "Host Anti-Microbial Response to Helicobacter Pylori Infection." *Molecular Immunology* 40 (7): 451–456.

- Gevensleben, Heidrun, Uwe-Jochen Göhring, Reinhard Büttner, Lukas C. Heukamp, Georg Kunz, Thomas Dimpfl, Christian Jackisch, Olaf Ortmann, Ute-Susann Albert, Richard Bender, Femke De Snoo, Oscar Krijgsman, Annuska M. Glas, Yavuz H. Ergönenc, Corinna Vogel, August Dykgers, Claus Langwieder, Martin Rees, and Tobias Anzeneder I. 2010. "Comparison of MammaPrint and TargetPrint Results with Clinical Parameters in German Patients with Early Stage Breast Cancer." *International Journal of Molecular Medicine* 26 (6): 837–843.
- Graham, Sheila V. 2012. "Human Papillomavirus : Gene Expression , Regulation and Prospects for Novel Diagnostic Methods and Antiviral Therapies" *Future Microbiology* 5 (10): 1493-1506.
- Grayson, W., L. F. Taylor, U. Allard, A. J. Tiltman, and H. A. Rhemtula. 2002. "Detection of Human Papillomavirus in Large Cell Neuroendocrine Carcinoma of the Uterine Cervix: A Study of 12 Cases." *Journal of Clinical Pathology* 55 (2): 108–14.
- Harwood, Catherine A., and Charlotte M. Proby. 2002. "Human Papillomaviruses and Non-Melanoma Skin Cancer." *Current Opinion in Infectious Diseases* 15 (2): 101–14.
- Haynes, Richard J., Jane E. McElveen, Harminder S. Dua, Patrick J. Tighe, and Janet Liversidg. 2000. "Expression of Human Beta-Defensins in Intraocular Tissues." *Investigative Ophthalmology & Visual Science* 41 (10): 3026–3031.
- Hill, John C., and Richard Maske. 1989. "Pathogenesis of Pterygium." *Eye* 3 (2): 218–226.
- Hindmarsh, Patrick L., and Laimonis A. Laimins. 2007. "Mechanisms Regulating Expression of the HPV 31 L1 and L2 Capsid Proteins and Pseudovirion Entry." *Virology Journal* 4 (1): 19.
- Hirst, Lawrence W. 2003. "The Treatment Of Pterygium." *Survey of Ophthalmology* 48 (2): 145–180.
- Hodge, David R., Elaine M. Hurt, and William L. Farrar. 2005. "The Role of IL-6 and STAT3 in Inflammation and Cancer." *European Journal of Cancer* 41 (16): 2502–2512.
- Hsiao, Ching-Hsi, Bor-Heng Lee, Kah-Wai Ngan, Wen-Yu Chuang, Ling Yeung, Lung-Kun Yeh, Hsin-Yuan Tan, David Hui-Kang, and Ken-Kuo Lin. 2010. "Presence of Human Papillomavirus in Pterygium in Taiwan." *Cornea* 29 (2): 123–127.
- Huang, Ling C., Daniele Jean, Rita J. Proske, Rose Y. Reins, and Alison M. McDermott. 2007. "Ocular Surface Expression and in Vitro Activity of Antimicrobial Peptides." *Current Eye Research* 32 (7–8): 595–609.

- Hubbard, Roger A. 2003. "Human Papillomavirus Testing Methods." *Archives of Pathology & Laboratory Medicine* 127 (8): 940–945.
- Hubert, Pascale, Ludivine Herman, Catherine Maillard, Jean-Hubert Caberg, Arjen Nikkels, Gerald Pierard, Jean-Marie Foidart, Agnes Noel, Jacques Boniver, and Philippe Delvenne. 2007. "Defensins Induce the Recruitment of Dendritic Cells in Cervical Human Papillomavirus-Associated (Pre)neoplastic Lesions Formed in Vitro and Transplanted in Vivo." *FASEB Journal* 21 (11): 2765–2775.
- Ikeda, Ai, Tohru Sakimoto, Jun Shoji, and Mitsuru Sawa. 2005. "Expression of α - and β -Defensins in Human Ocular Surface Tissue." *Japanese Journal of Ophthalmology* 49 (2): 73–78.
- Jarczak, Justyna, Ewa M. Kościuczuk, Paweł Lisowski, Nina Strzałkowska, Artur Józwiak, Jarosław Horbańczuk, Józef Krzyżewski, Lech Zwierzchowski, and Emilia Bagnicka. 2013. "Defensins: Natural Component of Human Innate Immunity." *Human Immunology* 74 (9): 1069–1079.
- Jia, Hong P., Brian C. Schutte, Andreas Schudy, Rose Linzmeier, Janet M. Guthmiller, Georgia K. Johnson, Brian F. Tack, Joseph P. Mitros, Andre Rosenthal, Tomas Ganz, and Paul B. McCray Jr. 2001. "Discovery of New Human Beta-Defensins Using a Genomics-Based Approach." *Gene* 263 (1): 211–218.
- Jin, Ge, Hameem I. Kawsar, Stanley A. Hirsch, Chun Zeng, Xun Jia, Zhimin Feng, Santosh K. Ghosh, Qing Yin Zheng, Aimin Zhou, Thomas M. McIntyre, and Aaron Weinberg. 2010. "An Antimicrobial Peptide Regulates Tumor-Associated Macrophage Trafficking via the Chemokine Receptor CCR2, a Model for Tumorigenesis." *PloS One* 5 (6): e10993.
- John, Minnie, Marla J. Keller, Ehsan H. Fam, Natalia Cheshenko, Kathleen Hogarty, Andrea Kasowitz, Sylvan Wallenstein, Maria J. Carlucci, Ana C. Tuyama, Wuyuan Lu, Mary E. Klotman, Robert I. Lehrer, and Betsy C. Herold. 2005. "Cervicovaginal Secretions Contribute to Innate Resistance to Herpes Simplex Virus Infection." *The Journal of Infectious Diseases* 192 (10): 1731–1740.
- Johnson, Katherine M., Rhonda C. Kines, Jeffrey N. Roberts, Douglas R. Lowy, John T. Schiller, and Patricia M. Day. 2009. "Role of Heparan Sulfate in Attachment to and Infection of the Murine Female Genital Tract by Human Papillomavirus." *Journal of Virology* 83 (5): 2067–2074.
- Johnston, Daniel S., Terry T. Turner, Joshua N. Finger, Tracy L. Owtscharuk, Gregory S. Kopf, and Scott A. Jelinsky. 2007. "Identification of Epididymis-Specific Transcripts in the Mouse and Rat by Transcriptional Profiling." *Asian Journal of Andrology* 9 (4): 522–527.

- Jong, Annemieke De, Mariëtte IE van Poelgeest, Jeanette M. van der Hulst, Jan Wouter Drijfhout, Gert Jan Fleuren, Cornelis JM Melief, Gemma Kenter, Rienk Offringa, and Sjoerd H. van der Burg. 2004. "Human Papillomavirus Type 16-Positive Cervical Cancer Is Associated with Impaired CD4 + T-Cell Immunity against Early Antigens E2 and E6." *Cancer Research* 64 (15): 5449-5455.
- Julio, Gemma, Sara Lluch, Pere Pujol, and Dolores Merindano. 2013. "Ocular Discomfort in Pterygium Patients." *Optometry and Vision Science* 90 (3): 269-274.
- Kadler, Karl E., Adele Hill, and Elizabeth G. Canty-laird. 2008. "Collagen Fibrillogenesis : Fibronectin , Integrins , and Minor Collagens as Organizers and Nucleators." *Current Opinion in Cell Biology* 20 (5): 495-501.
- Karcioglu, Zeynel A., and Tawfik M. Issa. 1997. "Human Papilloma Virus in Neoplastic and Non-Neoplastic Conditions of the External Eye." *The British Journal of Ophthalmology* 81 (7): 595-598.
- Kawauchi, K., A. Yagihashi, N. Tsuji, N. Uehara, D. Furuya, D. Kobayashi, and N. Watanabe. 2006. "Human Beta-Defensin-3 Induction in H. Pylori-Infected Gastric Mucosal Tissues." *World Journal of Gastroenterology* 12 (36): 5793-5797.
- Kawsar, Hameem I., Aaron Weinberg, Stanley A. Hirsch, Andrea Venizelos, Scott Howell, Bin Jiang, and Ge Jin. 2009. "Overexpression of Human Beta-Defensin-3 in Oral Dysplasia: Potential Role in Macrophage Trafficking." *Oral Oncology* 45 (8): 696-702.
- Kesting, Marco R., Denys J. Loeffelbein, Rafael J. Hasler, Klaus-Dietrich Wolff, Andrea Rittig, Matthias Schulte, Tobias Hirsch, Stefan Wagenpfeil, Frank Jacobsen, and Lars Steinstraesser. 2009. "Expression Profile of Human Beta-Defensin 3 in Oral Squamous Cell Carcinoma." *Cancer Investigation* 27 (5): 575-581.
- Kim, Kyoung W., Soo H. Park, Sung W. Wee, and Jae C. Kim. 2013. "Overexpression of Angiogenin in Pterygium Body Fibroblasts and Its Association with Proliferative Potency." *Investigative Ophthalmology and Visual Science* 54 (9): 6355-6362.
- Kim, Tae H., Yeoun S. Chun, and Jae C. Kim. 2013. "The Pathologic Characteristics of Pingueculae on Autofluorescence Images." *Korean Journal of Ophthalmology* 27 (6): 416-420.
- Klotman, Mary E., and Theresa L. Chang. 2006. "Defensins in Innate Antiviral Immunity." *Nature Reviews. Immunology* 6 (6): 447-456.

- kwok, L. Stephen, and Minas T. Coroneo. 1994 “A model for pterygium formation.” *Cornea* 13 (3): 219-224.
- Lagunas-Martínez, A., V. Madrid-Marina, and P. Gariglio. 2010. “Modulation of Apoptosis by Early Human Papillomavirus Proteins in Cervical Cancer.” *Biochimica et Biophysica Acta - Reviews on Cancer* 1805 (1): 6–16.
- Le Borgne, Sylvie, Michèle Février, Christian Callebaut, Steven P. Lee, and Yves Rivièrè. 2000. “CD8(+)-Cell Antiviral Factor Activity Is Not Restricted to Human Immunodeficiency Virus (HIV)-Specific T Cells and Can Block HIV Replication after Initiation of Reverse Transcription.” *Journal of Virology* 74 (10): 4456–4464.
- Lee, Seung-Bum, Baichuan Li, Shuangxia Jin, and Henry Daniell. 2011. “Expression and Characterization of Antimicrobial Peptides Retrocyclin-101 and Protegrin-1 in Chloroplasts to Control Viral and Bacterial Infections.” *Plant Biotechnology Journal* 9 (1): 100–115.
- Leibovich, S. Joseph, Peter J. Polverini, H. Michael Shepard, David M. Wiseman, Vera Shively, and Nureddin Nuseir. 1987. “Macrophage-Induced Angiogenesis Is Mediated by Tumour Necrosis Factor-[Alpha].” *Nature* 329 (6140): 630–632.
- Levy, Hara, Benjamin A. Raby, Stephen Lake, Kelan G. Tantisira, David Kwiatkowski, Ross Lazarus, Edwin K. Silverman, Brent Richter, Walter T. Klimecki, Donata Vercelli, Fernando D. Martinez, and Scott T. Weiss. 2005. “Association of Defensin Beta-1 Gene Polymorphisms with Asthma.” *The Journal of Allergy and Clinical Immunology* 115 (2): 252–258.
- Liu, Lide, Chengquan Zhao, Henry HQ Heng, and Tomas Ganz. 1997. “The Human β -Defensin-1 and α -Defensins Are Encoded by Adjacent Genes: Two Peptide Families with Differing Disulfide Topology Share a Common Ancestry.” *Genomics* 43 (3): 316–320.
- Liu, Lei, Jingyang Wu, Jin Geng, Zhe Yuan, and Desheng Huang. 2013. “Geographical Prevalence and Risk Factors for Pterygium: A Systematic Review and Meta-Analysis.” *British Medical Journal* 3 (11): e003787.
- Livak, Kenneth J., and Thomas D. Schmittgen. 2001. “Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method.” *Methods* 25 (4): 402–408.
- Livezeanu, C., Monica M. Crăițoiu, Rodica Mănescu, Carmen Mocanu, and Ștefania Crăițoiu. 2011. “Angiogenesis in the Pathogenesis of Pterygium.” *Romanian Journal of Morphology and Embryology* 52 (3): 837–844.

- Longworth, Michelle S., and Laimonis A. Laimins. 2004. "Pathogenesis of Human Papillomaviruses in Differentiating Epithelia Pathogenesis of Human Papillomaviruses in Differentiating Epithelia." *Microbiology and Molecular Biology Reviews* 68 (2): 362–372.
- Lucas, Robyn M., Anthony J. McMichael, Bruce K. Armstrong, and Wayne T. Smith. 2008. "Estimating the Global Disease Burden due to Ultraviolet Radiation Exposure." *International Journal of Epidemiology* 37 (3): 654–667.
- Machado, Lee R., and Barbara Ottolini. 2015. "An Evolutionary History of Defensins: A Role for Copy Number Variation in Maximizing Host Innate and Adaptive Immune Responses." *Frontiers in Immunology* 6 (3): 1–9.
- Makino, Yuichi, Hiroshi Nakamura, Eiji Ikeda, Kei Ohnuma, Kenji Yamauchi, Yutaka Yabe, Lorenz Poellinger, Yasunori Okada, Chikao Morimoto, and Hiroshi Tanaka. 2003. "Hypoxia-Inducible Factor Regulates Survival of Antigen Receptor-Driven T Cells." *Journal of Immunology* 171 (12): 6534–6540.
- Malumbres, Marcos, and Mariano Barbacid. 2009. "Cell Cycle, CDKs and Cancer: A Changing Paradigm" *Nature Reviews Cancer* 9 (3): 153-166.
- Mammas, Ioannis N., George Sourvinos, Apostolos Zaravinos, and Demetrios A. Spandidos. 2011. "Vaccination against Human Papilloma Virus (HPV): Epidemiological Evidence of HPV in Non-Genital Cancers." *Pathology and Oncology Research* 17 (1): 103–109.
- Mammas, Ioannis N., Demetrios A. Spandidos, and George Sourvinos. 2014. "Genomic Diversity of Human Papillomaviruses (HPV) and Clinical Implications: An Overview in Adulthood and Childhood." *Infection, Genetics and Evolution* 21 (1): 220–226.
- Mantovani, Fiamma, and Lawrence Banks. 2001. "The Human Papillomavirus E6 Protein and Its Contribution to Malignant Progression." *Oncogene* 20 (54): 7874–7887.
- Marcovich, Arie L., Yair Morad, Judith Sandbank, Monica Huszar, Mordechai Rosner, Ayala Pollack, Mehrdad Herbert, and Yaron Bar-Dayan. 2002. "Angiogenesis in Pterygium: Morphometric and Immunohistochemical Study." *Current Eye Research* 25 (1): 17–22.
- Mashino, Kohjiro, Noriaki Sadanaga, Hiroshi Yamaguchi, Fumiaki Tanaka, Mitsuhiro Ohta, Kenji Shibuta, Hiroshi Inoue, and Masaki Mori. 2002. "Expression of Chemokine Receptor CCR7 Is Associated with Lymph Node Metastasis of Gastric Carcinoma." *Cancer Research* 62 (10): 2937–2941.

- Masters, Jordan S., and David J. Harris. 2015. "Low Recurrence Rate of Pterygium after Excision with Conjunctival Limbal Autograft: A Retrospective Study with Long-Term Follow-Up." *Cornea* 34 (12): 1569–1572.
- Matsushita, Ikumi, Kyoko Hasegawa, Koh Nakata, Kazuki Yasuda, Katsushi Tokunaga, and Naoto Keicho. 2002. "Genetic Variants of Human Beta-Defensin-1 and Chronic Obstructive Pulmonary Disease." *Biochemical and Biophysical Research Communications* 291 (1): 17–22.
- Maxwell, A. I., G. M. Morrison, and J. R. Dorin. 2003. "Rapid Sequence Divergence in Mammalian Beta-Defensins by Adaptive Evolution." *Molecular Immunology* 40 (7): 413–421.
- Maxwell, Jessica H., Jennifer R. Grandis, and Robert L. Ferris. 2015. "HPV-Associated Head and Neck Cancer: Unique Features of Epidemiology and Clinical Management," *Annual Review of Medicine* 67 (2016): 91-101.
- Mayor, Roberto, and Sandrine Etienne-manneville. 2016. "The Front and Rear of Collective Cell Migration." *Nature Reviews. Molecular Cell Biology* 17 (2): 97.
- McDermott, Alison M. 2004. "Defensins and Other Antimicrobial Peptides at the Ocular Surface." *The Ocular Surface* 2 (4): 229–247.
- McDermott, Alison M. 2009. "The Role of Antimicrobial Peptides at the Ocular Surface." *Ophthalmic Research* 41 (2): 60–75.
- McLaughlin-Drubin, Margaret E. 2015. "Human Papillomaviruses and Non-Melanoma Skin Cancer." *Seminars in Oncology* 42 (2): 284–290.
- McNamara, Nancy A., Rajana Van, Oren S. Tuchin, and Suzanne M. Fleiszig. 1999. "Ocular Surface Epithelia Express mRNA for Human Beta Defensin-2." *Experimental Eye Research* 69 (5): 483–490.
- Meloni, Marisa, Barbara De Servi, Daniela Marasco, and Salvatore Del Prete. 2011. "Molecular Mechanism of Ocular Surface Damage: Application to an in Vitro Dry Eye Model on Human Corneal Epithelium." *Molecular Vision* 17 (1): 113–126.
- Melsheimer, Peter, Svetlana Vinokurova, Nicolas Wentzensen, Gunther Bastert, and Magnus von Knebel Doeberitz. 2004. "DNA Aneuploidy and Integration of Human Papillomavirus Type 16 e6/e7 Oncogenes in Intraepithelial Neoplasia and Invasive Squamous Cell Carcinoma of the Cervix Uteri." *Clinical Cancer Research* 10 (9): 3059–3063.

- Mohammed, Imran, Hanif Suleman, Ahmad M. Otri, Bina B. Kulkarni, Peng Chen, Andrew Hopkinson, and Harminder S. Dua. 2010. "Localization and Gene Expression of Human β -Defensin 9 at the Human Ocular Surface Epithelium." *Investigative Ophthalmology & Visual Science* 51 (9): 4677.
- Mohri, Y., T. Mohri, W. Wei, Y. J. Qi, A. Martin, C. Miki, M. Kusunoki, D. G. Ward, and P. J. Johnson. 2009. "Identification of Macrophage Migration Inhibitory Factor and Human Neutrophil Peptides 1-3 as Potential Biomarkers for Gastric Cancer." *British Journal of Cancer* 101 (2): 295–302.
- Moreau, Frédérique, Rachid Fetouchi, Isabelle Micalessi, Valérie Brejeon, Nathalie Bacon, Geert Jannes, Catherine Le Pendeven, Bouchra Lekbaby, Dina Kremsdorf, Jean L. Saint Guily, and Patrick Soussan. 2013. "Detection and Genotyping of Human Papillomavirus by Real-Time PCR Assay." *Journal of Clinical Virology* 56 (3): 328-333.
- Mott, Joni D., and Zena Werb. 2004. "Regulation of Matrix Biology by Matrix Metalloproteinases." *Current opinion in cell biology* 16 (5): 558-564.
- Musumeci, Giuseppe, Maria L. Carnazza, Rosalia Leonardi, and Carla Loreto. 2012. "Expression of β -Defensin-4 In 'an in Vivo and Ex Vivo Model' of Human Osteoarthritic Knee Meniscus." *Knee Surgery, Sports Traumatology, Arthroscopy* 20 (2): 216–222.
- Nelson, J. Daniel. 1988. "Impression Cytology." *Cornea* 7 (1): 71–81.
- Niyonsaba, François, Akimasa Someya, Michimasa Hirata, Hideoki Ogawa, and Isao Nagaoka. 2001. "Evaluation of the Effects of Peptide Antibiotics Human Beta-Defensins-1/-2 and LL-37 on Histamine Release and Prostaglandin D(2) Production from Mast Cells." *European Journal of Immunology* 31 (4): 1066–1075.
- Niyonsaba, Francois, Hiroko Ushio, Mutsuko Hara, Hidenori Yokoi, Mitsutoshi Tominaga, Kenji Takamori, Naoki Kajiwara, Hirohisa Saito, Isao Nagaoka, Hideoki Ogawa and Ko Okumura. 2010. "Antimicrobial Peptides Human Beta-Defensins and Cathelicidin LL-37 Induce the Secretion of a Pruritogenic Cytokine IL-31 by Human Mast Cells." *Journal of Immunology* 184 (7): 3526–3534.
- Nubile, Mario, Claudia Curcio, Manuela Lanzini, Roberta Calienno, Manuela Iezzi, Alessandra Mastropasqua, Marta Di Nicola, and Leonardo Mastropasqua. 2013. "Expression of CREB in primary pterygium and correlation with cyclin D1, ki-67, MMP7, p53, p63, Survivin and Vimentin." *Ophthalmic Research* 50 (2): 99-107.

- Ong, Peck Y., Takaaki Ohtake, Corinne Brandt, Ian Strickland, Mark Boguniewicz, Tomas Ganz, Richard L. Gallo, and Donald YM Leung. 2002. "Endogenous Antimicrobial Peptides and Skin Infections in Atopic Dermatitis." *The New England Journal of Medicine* 347 (15): 1151–1160.
- Otlu, Baris, Sinan Emre, Peykan Turkuoglu, Selim Doganay, and Riza Durmaz. 2009. "Investigation of Human Papillomavirus and Epstein-Barr Virus DNAs in Pterygium Tissue." *European Journal of Ophthalmology* 19 (2): 175–179.
- Otri, Ahmad M., Imran Mohammed, Mouhamed A. Al-Aqaba, Usama Fares, Chen Peng, Andrew Hopkinson, and Harminder S. Dua. 2012. "Variable Expression of Human Beta Defensins 3 and 9 at the Human Ocular Surface in Infectious Keratitis." *Investigative Ophthalmology & Visual Science* 53 (2): 757-761.
- Otrock, Zaher K., Rami AR Mahfouz, Jawad A. Makarem, and Ali I. Shamseddine. 2007. "Understanding the Biology of Angiogenesis: Review of the Most Important Molecular Mechanisms" *Blood Cells, Molecules, and Diseases* 39 (2): 212-220.
- Otte, J-M., H. M. Neumann, S. Brand, H. Schrader, W. E. Schmidt, and F. Schmitz. 2009. "Expression of Beta-Defensin 4 Is Increased in Human Gastritis." *European Journal of Clinical Investigation* 39 (2): 126–138.
- Owen, James D., Robert Strieter, Marie Burdick, Hamid Haghnegahdar, Lillian Nanney, Rebecca Shattuck-Brandt, and Ann Richmond. 1997. "Enhanced Tumor-Forming Capacity for Immortalized Melanocytes Expressing Melanoma Growth Stimulatory Activity/growth-Regulated Cytokine Beta and Gamma Proteins." *International Journal of Cancer* 73 (1): 94–103.
- Ozkurt, Yelda B., Ozkan Kocams, Arzu T. Comez, Burcu Uslu, and Omer K. Dogan. 2009. "Treatment of Primary Pterygium." *Optometry and Vision Science* 86 (10): 1178–1181.
- Patel, Tejas, L. Katie Morrison, Peter Rady, and Stephen Tying. 2010. "Epidermodysplasia Verruciformis and Susceptibility to HPV." *Disease Markers* 29 (3–4): 199–206.
- Patil, Sneha P., Sonia J. Sodhi, Suraj D. Tambe, Viral Gada, and Deepak Vikhe. 2016. "Viruses in Oral Squamous Cell Carcinoma: A Review" *Pravara Medical Review* 8 (1): 4–7.
- Pazgier, M., D. M. Hoover, D. Yang, W. Lu, and J. Lubkowski. 2006. "Human β -Defensins." *Cellular and Molecular Life Sciences* 63 (11): 1294–1313.
- Piras, F., P. S. Moore, J. Ugalde, M. T. Perra, A. Scarpa, and P. Sirigu. 2003. "Detection of Human Papillomavirus DNA in Pterygia from Different Geographical Regions." *British Journal of Ophthalmology* 87 (7): 864–866.

- Peyton, Cheri L., Patti E. Gravitt, William C. Hunt, Rosalina S. Hundley, Meifen Zhao, Raymond J. Apple, and Cosette M. Wheeler. 2001. "Determinants of Genital Human Papillomavirus Detection in a US Population." *The Journal of Infectious Diseases* 183 (11): 1554–1564.
- Prado-Montes de Oca, Ernesto. 2010. "Human Beta-Defensin 1: A Restless Warrior against Allergies, Infections and Cancer." *The International Journal of Biochemistry & Cell Biology* 42 (6): 800–804.
- Premratanachai, Porntip, S. Joly, G. K. Johnson, P. B. McCray, H. P. Jia, and Janet M. Guthmiller. 2004. "Expression and Regulation of Novel Human β -Defensins in Gingival Keratinocytes." *Oral Microbiology and Immunology* 19 (2): 111–117.
- Proud, David, Scherer P. Sanders, and Shahina Wiehler. 2004. "Human Rhinovirus Infection Induces Airway Epithelial Cell Production of Human Beta-Defensin 2 Both in Vitro and in Vivo." *Journal of Immunology* 172 (7): 4637–4645.
- Putsep, Katrin, Goran Carlsson, Hans G. Boman, and Mats Andersson. 2002. "Deficiency of Antibacterial Peptides in Patients with Morbus Kostmann: An Observation Study." *Lancet* 360 (9340): 1144–1149.
- Quinones-Mateu, Miguel E., Michael M. Lederman, Zhimin Feng, Bikram Chakraborty, Jan Weber, Hector R. Rangel, Michael L. Marotta, Muneer Mirzaa, Bin Jiangc, Patti Kisera, Kathy Medvikb, Scott F. Siegb and Aaron Weinberg. 2003. "Human Epithelial Beta-Defensins 2 and 3 Inhibit HIV-1 Replication." *AIDS* 17 (16): F39-48.
- Rachmiel, R., H. Leiba, and S. Levartovsky. 1995. "Results of Treatment with Topical Mitomycin C 0.02% Following Excision of Primary Pterygium." *British Journal Ophthalmology* 79 (3): 233–236.
- Ramasundara, Malith, Steven T. Leach, Daniel A. Lemberg, and Andrew S. Day. 2009. "Defensins and Inflammation: The Role of Defensins in Inflammatory Bowel Disease." *Journal of Gastroenterology and Hepatology* 24 (2): 202–208.
- Rector, Annabel, and Marc Van Ranst. 2013. "Animal Papillomaviruses." *Virology* 445 (1–2): 213–223.
- Richmond, A., and H. G. Thomas. 1986. "Purification of Melanoma Growth Stimulatory Activity." *Journal of Cellular Physiology* 129 (3): 375–384.
- Salvatore, Mirella, Adolfo Garcia-Sastre, Piotr Ruchala, Robert I. Lehrer, Theresa Chang, and Mary E. Klotman. 2007. "Alpha-Defensin Inhibits Influenza Virus Replication by Cell-Mediated Mechanism(s)." *The Journal of Infectious Diseases* 196 (6): 835–843.

- Sapp, Martin, Claudia Fligge, Ingrid Petzak, J. Robin Harris, and Rolf E. Streeck. 1998. "Papillomavirus Assembly Requires Trimerization of the Major Capsid Protein by Disulfides between Two Highly Conserved Cysteines." *Journal of Virology* 72 (7): 6186–6189.
- Saw, Seang-Mei, and Donald Tan. 1999. "Pterygium: Prevalence, Demography and Risk Factors." *Ophthalmic Epidemiology* 6 (3): 219–228.
- Scheffner, Martin, Bruce A. Werness, Jon M. Huibregtse, Arnold J. Levine, and Peter M. Howley. 2017. "The E6 Oncoprotein Encoded by Human Papillomavirus Types 16 and 18 Promotes the Degradation of p53." *Cell* 63 (6): 1129–1136.
- Schellini, Silvana A., Erika Hoyama, Claudia A. Shiratori, Regina H. Sakamoto, and João M. Grisi Candeias. 2006. "Lack of Papillomavirus (HPV) in Pterygia of a Brazilian Sample." *Arquivos Brasileiros de Oftalmologia* 69 (4): 519–521.
- Schiller, John T., Patricia M. Day, and Rhonda C. Kines. 2010. "Current Understanding of the Mechanism of HPV Infection." *Gynecologic Oncology* 118 (1): S12-S17.
- Schmitt, Markus, I. G. Bravo, Peter JF Snijders, Lutz Gissmann, Michael Pawlita, and Tim Waterboer. 2006. "Bead-Based Multiplex Genotyping of Human Papillomaviruses." *Journal of Clinical Microbiology* 44 (2): 504–512.
- Schutte, Brian C., Joseph P. Mitros, Jennifer A. Bartlett, Jesse D. Walters, Hong Peng Jia, Michael J. Welsh, Thomas L. Casavant, and Paul B. McCray. 2002. "Discovery of Five Conserved Beta -Defensin Gene Clusters Using a Computational Search Strategy." *Proceedings of the National Academy of Sciences of the United States of America* 99 (4): 2129–2133.
- Sekelj, Sandra, Iva Dekaris, Edita Kondza-Krstonijević, Nikica Gabrić, Jurica Predović, and Sanja Mitrović. 2007. "Ultraviolet Light and Pterygium." *Collegium Antropologicum* 31 (1): 45–47.
- Selsted, Michael E., and Andre J. Ouellette. 2005. "Mammalian Defensins in the Antimicrobial Immune Response." *Nature Immunology* 6 (6): 551–557.
- Semple, Colin AM, Mark Rolfe, and Julia R. Dorin. 2003. "Duplication and Selection in the Evolution of Primate Beta-Defensin Genes." *Genome Biology* 4 (5): R31.
- Shah, Syed Imtiaz A., Shujaat A. Shah, and Partab Rai. 2016. "Factors Associated with Pterygium Based on History and Clinical Examination of Patients in Pakistan." *Journal of Current Ophthalmology* 28 (2): 91-92

- Shayegan, Mohammad R., Mohammad R. Khakzad, Hamid Gharaee, A-Reza Varasteh, and Mojtaba Sankian. 2016. "Evaluation of Transforming Growth Factor-beta1 Gene Expression in Pterygium Tissue of Atopic Patients." *Journal of the Chinese Medical Association* 79 (10): 565–569.
- Sherwin, Justin C., Alex W. Hewitt, Lisa S. Kearns, Lyn R. Griffiths, David A. MacKey, and Minas T. Coroneo. 2013. "The Association between Pterygium and Conjunctival Ultraviolet Autofluorescence: The Norfolk Island Eye Study." *Acta Ophthalmologica* 91 (4): 363–370.
- Shusko, Alexander, and John A. Hovanesian. 2016. "Pterygium Excision with Conjunctival Autograft and Subconjunctival Amniotic Membrane as Antirecurrence Agents." *Canadian Journal of Ophthalmology / Journal Canadien d'Ophthalmologie* 51 (6): 412-416.
- Singh, R., A. Joseph, T. Umapathy, N. L. Tint, and H. S. Dua. 2005. "Impression Cytology of the Ocular Surface." *British Journal of Ophthalmology* 89 (12): 1655–1659.
- Sjö, Nicolai C., Christian von Buchwald, Jan U. Prause, Bodil Norrild, Troels Vinding, and Steffen Heegaard. 2007. "Human Papillomavirus and Pterygium. Is the Virus a Risk Factor?" *British Journal of Ophthalmology* 91 (8): 1016–1018.
- Smith, Jessica L., Samuel K. Campos, Angela Wandinger-Ness, and Michelle A. Ozbun. 2008. "Caveolin-1-Dependent Infectious Entry of Human Papillomavirus Type 31 in Human Keratinocytes Proceeds to the Endosomal Pathway for pH-Dependent Uncoating." *Journal of Virology* 82 (19): 9505–9512.
- Smithrithee, Rithee, François Niyonsaba, Chanisa Kiatsurayanon, Hiroko Ushio, Shigaku Ikeda, Ko Okumura, and Hideoki Ogawa. 2015. "Human β -Defensin-3 Increases the Expression of Interleukin-37 through CCR6 in Human Keratinocytes." *Journal of Dermatological Science* 77 (1): 46–53.
- Suarez-Carmona, Meggy, Pascale Hubert, Philippe Delvenne, and Michael Herfs. 2015. "Defensins: 'Simple' Antimicrobial Peptides or Broad-Spectrum Molecules?" *Cytokine & Growth Factor Reviews* 26 (3): 361–370.
- Sun, Carrie Q., Rebecca Arnold, Carina Fernandez-Golarz, Amanda B. Parrish, Tara Almekinder, Ju He, Shuk-mei Ho, Pavel Svoboda, Jan Pohl, Fray F. Marshall and John A. Petros. 2006. "Human Beta-Defensin-1, a Potential Chromosome 8p Tumor Suppressor: Control of Transcription and Induction of Apoptosis in Renal Cell Carcinoma." *Cancer Research* 66 (17): 8542–8549.

- Sun, Lingling, Catherine M. Finnegan, Tina Kish-Catalone, Robert Blumenthal, Paolo Garzino-Demo, Gian M. La Terra Maggiore, Sid Berrone, Carol Kleinman, Zhibin Wu, Sayed Abdelwahab, Wuyuan Lu, and Alfredo Garzino-Demo. 2005. "Human Beta-Defensins Suppress Human Immunodeficiency Virus Infection: Potential Role in Mucosal Protection." *Journal of Virology* 79 (22): 14318–14329.
- Surviladze, Zurab, Agnieszka Dziduszko, and Michelle A. Ozbun. 2012. "Essential Roles for Soluble Virion-Associated Heparan Sulfonated Proteoglycans and Growth Factors in Human Papillomavirus Infections." *PLoS Pathogens* 8 (2): e1002519.
- Talghini, Shahla, and Abdollah Shenasi. 2013. "Concomitant Examination of Inflammation and Angiogenesis in the Pathogenesis of Primary Moderate Pterygium in a Well-Designed Case-Control Study." *Pakistan Journal of Biological Sciences* 16 (19): 1046–1050.
- Tan, Donald TH, Wen Ying Tang, Yan Ping Liu, Hak-Su Goh, and Duncan R. Smith. 2000. "Apoptosis and Apoptosis Related Gene Expression in Normal Conjunctiva and Pterygium." *British Journal of Ophthalmology* 84 (2): 212–216.
- Tang, Yi-Quan, Jun Yuan, George Ösapay, Klara Ösapay, Dat Tran, Christopher J. Miller, Andre J. Ouellette, and Michael E. Selsted. 1999. "A Cyclic Antimicrobial Peptide Produced in Primate Leukocytes by the Ligation of Two Truncated α -Defensins." *Science* 286 (5439): 498–502.
- Torres, Jenice, Amalia Enríquez-de-Salamanca, Itziar Fernández, Maria Teresa Rodríguez-Ares, Maria J. Quadrado, Joaquim Murta, José M. Benítez del Castillo, Michael E. Stern, and Margarita Calonge. 2011. "Activation of MAPK Signaling Pathway and NF- κ B Activation in Pterygium and Ipsilateral Pterygium-Free Conjunctival Specimens," *Investigative Ophthalmology & Visual Science* 49 (13): 6037-6037.
- Tsai, Yi-yu, Da-tian Bau, Chun-chi Chiang, Ya-wen Cheng, Sung-huei Tseng, and Fuu-jen Tsai. 2007. "Pterygium and Genetic Polymorphism of DNA Double Strand Break Repair Gene Ku70," *Molecular Vision* 13: 1436-1440.
- Tsai, Yi-Yu, Chi-Chung Chang, Chun-Chi Chiang, Kun-Tu Yeh, Pei-Liang Chen, Chi-Huang Chang, Ming-Chih Chou, Huei Lee, and Ya-Wen Cheng. 2009. "HPV Infection and p53 Inactivation in Pterygium." *Molecular Vision* 15: 1092.
- Valore, Erika V., Edith Martin, Sylvia SL Harwig, and Tomas Ganz. 1996. "Intramolecular Inhibition of Human Defensin HNP-1 by Its Propiece." *Journal of Clinical Investigation* 97 (7): 1624–1629.

- Valore, Erika V., Dorothy J. Wiley, and Tomas Ganz. 2006. "Reversible Deficiency of Antimicrobial Polypeptides in Bacterial Vaginosis." *Infection and Immunity* 74 (10): 5693–5702.
- Varinli, S., I. Varinli, Erkisi M. Köksal, and F. Doran. 1994. "Human Papillomavirus in Pterygium." *The Central African Journal of Medicine* 40 (1): 24–26.
- Varssano, David, Hadas Shalev, Moshe Lazar, and Naomi Fischer. 2013. "Pterygium Excision with Conjunctival Autograft: True Survival Rate Statistics." *Cornea* 32 (9): 1243–1250.
- Venceslau, Emanuella M., Mauro M. Bezerra, Anna Carolina, Mota Lopes, Alexandre Sherlley, Casimiro Onofre, Claudia M. De Melo, Fabiana Botelho, and De Miranda Onofre. 2014. "HPV Detection Using Primers MY09 / MY11 and GP5 + / GP6 + in Patients with Cytologic and / or Colposcopic Changes," *Jornal Brasileiro de Patologia e Medicina Laboratorial* 50 (4): 280-285.
- Venkataraman, Nitya, Amy L. Cole, Pavel Svoboda, Jan Pohl, and Alexander M. Cole. 2005. "Cationic Polypeptides Are Required for Anti-HIV-1 Activity of Human Vaginal Fluid." *Journal of Immunology* 175 (11): 7560–7567.
- Vora, Puja, Adrienne Youdim, Lisa S. Thomas, Masayuki Fukata, Samuel Y. Tesfay, Katie Lukasek, Kathrin S. Michelsen, Akihiro Wada, Toshiya Hirayama, Moshe Arditi and Maria T. Abreu. 2004. "Beta-Defensin-2 Expression Is Regulated by TLR Signaling in Intestinal Epithelial Cells." *Journal of Immunology* 173 (9): 5398–5405.
- Vordenbäumen, Stefan, Daniel Timm, Ellen Bleck, Jutta Richter, Rebecca Fischer-Betz, Gamal Chehab, Oliver Sander, and Matthias Schneider. 2011. "Altered Serum Levels of Human Neutrophil Peptides (HNP) and Human Beta-Defensin 2 (hBD2) in Wegener's Granulomatosis." *Rheumatology International* 31 (9): 1251–1254.
- Walboomers, Jan MM, Marcel V. Jacobs, M. Michele Manos, F. Xavier Bosch, J. Alain Kummer, Keerti V. Shah, Peter JF Snijders, Julian Peto, C. J. L. M. Meijer, and Nubia Munoz. 1999. "Human Papillomavirus Is a Necessary Cause of Invasive Cervical Cancer Worldwide." *Journal of Pathology* 189 (1): 12–19.
- Walmsley, Sarah R., Neda Farahi, Carole Peyssonnaud, Randall S. Johnson, Thorsten Cramer, Anastasia Sobolewski, Alison M. Condliffe, Andrew S. Cowburn, Nicola Johnson, and Edwin R. Chilvers. 2005. "Hypoxia-Induced Neutrophil Survival Is Mediated by HIF-1alpha-Dependent NF-kappaB Activity." *The Journal of Experimental Medicine* 201 (1): 105–115.

- Wang, Jun, Xin Zhang, Sufi M. Thomas, Jennifer R. Grandis, Alan Wells, Zhuo Georgia Chen, and Robert L. Ferris. 2005. "Chemokine Receptor 7 Activates Phosphoinositide-3 Kinase-Mediated Invasive and Prosurvival Pathways in Head and Neck Cancer Cells Independent of EGFR." *Oncogene* 24 (38): 5897–5904.
- Wang, Xiao-Fang, Rui-Ming Cao, Jing Li, Jing Wu, Sheng-Mei Wu, and Tong-Xin Chen. 2014. "Identification of Sociodemographic and Clinical Factors Associated with the Levels of Human β -Defensin-1 and Human β -Defensin-2 in the Human Milk of Han Chinese." *The British Journal of Nutrition* 111 (5): 867–874.
- Wang, Yong-Sheng, Dan Li, Hua-Shan Shi, Yan-Jun Wen, Li Yang, Ning Xu, Xian-Cheng Chen, Xiang Chen, Ping Chen, Jiong Li, Hong-xin Deng, Chun-ting Wang, Gang Xie, Shan Huang, Yong-qiu Mao, Li-juan Chen, Xia Zhao and Yu-quan Wei. 2009. "Intratumoral Expression of Mature Human Neutrophil Peptide-1 Mediates Antitumor Immunity in Mice." *Clinical Cancer Research* 15 (22): 6901–6911.
- Wehkamp, Jan, Juergen Harder, Michael Weichenthal, Oliver Mueller, Klaus R. Herrlinger, Klaus Fellermann, Jens M. Schroeder, and Eduard F. Stange. 2003. "Inducible and Constitutive Beta-Defensins Are Differentially Expressed in Crohn's Disease and Ulcerative Colitis." *Inflammatory Bowel Diseases* 9 (4): 215–223.
- Wehkamp, Jan, Nita H. Salzman, Edith Porter, Sabine Nuding, Michael Weichenthal, Robert E. Petras, Bo Shen, Elke Schaeffeler, Matthias Schwab, Rose Linzmeier, Ryan W. Feathers, Hiutung Chu, Heriberto Lima, Jr, Klaus Fellermann, Tomas Ganz, Eduard F. Stange, and Charles L. Bevins. 2005. "Reduced Paneth Cell Alpha-Defensins in Ileal Crohn's Disease." *Proceedings of the National Academy of Sciences of the United States of America* 102 (50): 18129–18134.
- Wehkamp, Jan, Guoxing Wang, Irmgard Kübler, Sabine Nuding, Alex Gregorieff, Anke Schnabel, Robert J. Kays, Klaus Fellermann, Oliver Burk, Matthias Schwab, Hans Clevers, Charles L. Bevins and Eduard F. Stange. 2007. "The Paneth Cell Alpha-Defensin Deficiency of Ileal Crohn's Disease Is Linked to Wnt/Tcf-4." *Journal of Immunology* 179 (5): 3109–3118.
- Weinstein, O., G. Rosenthal, H. Zirkin, T. Monos, T. Lifshitz, and S. Argov. 2002. "Overexpression of p53 Tumor Suppressor Gene in Pterygia." *Eye* 16 (5): 619–621.
- Welti, Jonathan, Sonja Loges, Stefanie Dimmeler, and Peter Carmeliet. 2013. "Recent Molecular Discoveries in Angiogenesis and Antiangiogenic Therapies in Cancer" *The Journal of Clinical Investigation* 123 (8): 3190–3200.

- Wen, Bo, Jed N. Lampe, Arthur G. Roberts, William M. Atkins, A. David Rodrigues, and Sidney D. Nelson. 2007. "Defensins and other antimicrobial peptides at the ocular surface." *The Ocular Surface* 2 (4): 229-247.
- Willoughby, Colin E., Diego Ponzin, Stefano Ferrari, Aires Lobo, Klara Landau, and Yadollah Omid. 2010. "Anatomy and Physiology of the Human Eye: Effects of Mucopolysaccharidoses Disease on Structure and Function - a Review." *Clinical and Experimental Ophthalmology* 38 (1): 2–11.
- Wilson, Carole L., Amy P. Schmidt, Emma Pirilä, Erika V. Valore, Nicola Ferri, Timo Sorsa, Tomas Ganz, and William C. Parks. 2009. "Differential Processing of α - and β -Defensin Precursors by Matrix Metalloproteinase-7 (MMP-7)." *Journal of Biological Chemistry* 284 (13): 8301–8311.
- Wilson, Sarah S., Mayim E. Wiens, and Jason G. Smith. 2013. "Antiviral Mechanisms of Human Defensins." *Journal of Molecular Biology* 425 (24): 4965–4980.
- Winter, Jochen, and Matthias Wenghoefer. 2012. "Human Defensins: Potential Tools for Clinical Applications." *Polymers* 4 (1): 691–709.
- Woods, Mark, Sharron Chow, Benjamin Heng, Wendy Glenn, Noel Whitaker, Dale Waring, Jenna Iwasenko, William Rawlinson, Denis Wakefield, and Nick Di Girolamo. 2015. "Detecting Human Papillomavirus in Ocular Surface Diseases Detecting Human Papillomavirus in Ocular Surface Diseases Detecting Human Papillomavirus in Ocular Surface Diseases," *Investigative Ophthalmology & Visual Science*, 54(13): 8069-8078.
- Wu, Chueh-Wei, Mei-Ling Peng, Ken-Tu Yeh, Yi-Yu Tsai, Chun-Chi Chiang, and Ya-Wen Cheng. 2016. "Corrigendum to 'Inactivation of p53 in Pterygium Influence miR-200a Expression Resulting in ZEB1/ZEB2 up-Regulation and EMT Processing.'" *Experimental Eye Research*. 146 (2016): 206–211.
- Wynn, Thomas A. 2009. "Cellular and molecular mechanisms of fibrosis." *The Journal of pathology* 214 (2): 199–210.
- Yamaguchi, Yasuhiro, Takahide Nagase, Ryosuke Makita, Shigetomo Fukuhara, Tetsuji Tomita, Takashi Tominaga, Hiroki Kurihara, and Yasuyoshi Ouchi. 2002. "Identification of Multiple Novel Epididymis-Specific β -Defensin Isoforms in Humans and Mice." *Journal of Immunology* 169 (5): 2516–2523.
- Yang, D., O. B. S. N. Chertov, S. N. Bykovskaia, Q. Chen, M. J. Buffo, J. Shogan, M. Anderson, J. M. Schröder, J. M. Wang, O. M. Z. Howard, and J. J. Oppenheim. 1999. "Beta-Defensins: Linking Innate and Adaptive Immunity through Dendritic and T Cell CCR6." *Science* 286 (5439): 525–528.
- Yang, De, Arya Biragyn, Larry W. Kwak, and Joost J. Oppenheim. 2002. "Mammalian Defensins in Immunity: More than Just Microbicidal." *Trends in Immunology* 23 (6): 291–296.

- Yang, Shaowei, Kui Ma, Zhijun Geng, Xiaoyan Sun, and Xiaobing Fu. 2015. "Oriented Cell Division: New Roles in Guiding Skin Wound Repair and Regeneration." *Bioscience Reports* 35 (6): e00280.
- Yoon, Chun-Sik, Kwang-Dong Kim, Sue-Nie Park, and Seon-Woo Cheong. 2001. "α6 Integrin Is the Main Receptor of Human Papillomavirus Type 16 VLP." *Biochemical and Biophysical Research Communications* 283 (3): 668–673.
- Zapata, Wildeman, Benigno Rodriguez, Jan Weber, Hernando Estrada, Miguel E. Quinones-Mateu, Peter A. Zimmerman, Michael M. Lederman, and Maria T. Rugeles. 2008. "Increased Levels of Human Beta-Defensins mRNA in Sexually HIV-1 Exposed but Uninfected Individuals." *Current HIV Research* 6 (6): 531–538.
- Zhou, Yu S., Sheila Webb, Laura Lettice, Steve Tardif, Fiona Kilanowski, Christine Tyrrell, Heather Macpherson, Fiona Semple, Peter Tennant, Tina Baker, Alan Hart, Paul Devenney, Paul Perry, Tracey Davey, Perdita Barran, Chris L. Barratt, and Julia R. Dorin. 2013. "Partial Deletion of Chromosome 8 B - Defensin Cluster Confers Sperm Dysfunction and Infertility in Male Mice" *PLoS Genetics* 9 (10): e1003826.
- Zur Hausen, Harald. 2002. "Papillomaviruses and Cancer: From Basic Studies to Clinical Application." *Nature Reviews.Cancer* 2 (5): 342–350.