Immunohistochemical Expression Of Topoisomerase IIα in Benign and Malignant Salivary Gland Tumours

Thesis

Submitted to Faculty of Oral and Dental Medicine, Cairo University in Partial fulfillment of the requirements for the Doctor Degree of Philosophy in Oral Pathology

Dina Soliman Khater
B.D.S., M.D.Sc.
Assistant Lecturer of Oral Pathology
Faculty of Oral & Dental Medicine,
Cairo University

Faculty of Oral & Dental Medicine, Cairo University

Supervisors

Prof. Dr. Heba Ahmed Farag

Professor of Oral Pathology
Faculty of Oral and Dental Medicine,
Cairo University

Dr. Hend Mohamed Waguih Mahmoud Salem

Lecturer of Oral Pathology
Faculty of Oral and Dental Medicine,
Cairo University

دراسة نسيجية كيميائية مناعية لانتشار توبوايزومريز 2-الفا في الاورام الحميدة و الخبيثة للغدد اللعابية

رسالة مقدمة الي كلية طب الفم و الأسنان جامعة القاهره توطئه للحصول علي درجة دكتوراة الفلسفة في العلوم الأساسيه لطب الأسنان (باثولوجيا الفم)

مقدمة من الطبيبة/ دينا سليمان خاطر المدرس المساعد بقسم باثولوجيا الفم كلية طب الفم و الأسنان جامعة القاهرة

قسم باثولوجيا الفم كلية طب الفم و الأسنان جامعة القاهرة

تحت اشراف

الأستاذ الدكتور/ هبه احمد فرج

استاذ باثولوجيا الفم كلية طب الفم و الأسنان جامعة القاهرة

الدكتور/ هند محمد وجيه محمود سالم مدرس باثولوجيا الفم كلية طب الفم و الأسنان جامعة القاهرة

بسم الله الرحمن الرحيم

﴿ وَقُل رَبِّ زِدْنِي عِلْماً ﴾

" صدق الله العظيم" سورة طه الآيه رقم 114



Acknowledgement

I am deeply grateful to Prof. Dr. *Heba Ahmed Farag*, Professor of Oral Pathology, Faculty of Oral and Dental Medicine, Cairo University, for her kind and close supervision, valuable advice, wise guidance, generous help and constant encouragement throughout this work.

I would like to express my deepest gratitude to Dr. Hend Mohamed Waguíh Mahmoud Salem, Lecturer of Oral Pathology, Faculty of Oral and Dental Medicine, Cairo University, for her faithful assistance, co-operation, willing support and constructive advice.

Finally, I wish to thank all my professors and colleagues in the Oral Pathology Department, Oral Pathology, Faculty of Oral and Dental Medicine, Cairo University, for their co-operation and facilities they offered during the course of this study.

Aim of the study

The aim of the present study is to determine the expression of DNA topoisomerase $II\alpha$ in benign and malignant salivary gland tumours and to correlate this expression with their histopathological features.

úi-List of Abbreviations

Abbreviations	Words
AgNoR	Silver nuclear organizer region
E. coli	Escherichia coli
EGFP	Enhanced green fluorescent protein
MEC	Mucoepidermoid carcinoma
N/C ratio	Nuclear/cytoplasmic ratio
PCNA	Proliferating cell nuclear antigen
PLGA	Polymorphous low grade adenocarcinoma
SCLC	Small cell lung cancer
Τορο ΙΙα	Topoisomerase IIα
WHO	World health organization

List of Contents

Chapter	Page
Introduction	1
Review of Literature	3
-History and classification	3 8
-Structure of type II topoisomerases -Biological functions of DNA topoisomerase II	15
-Regulation of topoisomerase II functions by phosphorylation	21
-Cellular locations of topoisomerase II	23
-Extranuclear expression of topoisomerase IIα	25 28
-DNA topoisomerase II targeting therapeutics	30
-Prognostic values of topoisomerase IIα -Immunohistochemical expression of topo IIα	32
Aim of the study	37
Materials and Method	38
Results	51
-Histopathological and Immunohistochemical findings	51
of topo IIα -Statistical analysis	90
Discussion	98
	112
Conclusion	113
Summary	115
References	117
Arabic summary	

ú-List of Tables

Table Number	Table Títle	Pages
Number		
Table (1)	Classification of topoisomerases	7
Table (2)	Histopathological diagnosis of the studied cases	39
Table (3)	Area percentage of topo IIα positive cells in benign and malignant salivary gland tumours	92
Table (4)	Immunostaining intensity of topo IIα positive cells in benign and malignant salivary gland tumours	93
Table (5)	Area percentage of topo IIα positive cells in different histological patterns of adenoid cystic carcinoma	94
Table (6)	LSD test to compare area percentage of topo IIα in adenoid cystic carcinoma	94
Table (7)	Immunostaining intensity of topo IIα positive cells in different histological patterns of adenoid cystic carcinoma	95
Table (8)	LSD test to compare immunostaining intensity of topo IIα positive cells in adenoid cystic carcinoma	95
Table (9)	Comparing the area percentage of topo IIα positive cells in pleomorphic adenoma and malignant pleomorphic adenoma	96
Table (10)	Comparing the immunostaining intensity of topo IIα positive cells in pleomorphic adenoma and malignant pleomorphic adenoma	96
Table (11)	Comparing the area percentage of topo IIa positive cells in low grade mucoepidermoid carcinoma and high grade mucoepidermoid carcinoma	97
Table (12)	Comparing the immunostaining intensity of topo IIa positive cells in low grade mucoepidermoid carcinoma and high grade mucoepidermoid carcinoma	97

ú-List of Figures

Figure	Figure Caption	Pages
Fígure Number		
Figure (1)	Action of E. coli DNA gyrase, a type II topoisomerase: introduction of negative supercoils.	3
Figure (2)	Sequence comparisons among eukaryotic type II topo- isomerases.	9
Figure (3)	Representation of the yeast DNA topoisomerase II dimer. The two crescent shaped monomers form a pair to make a heart shaped dimer with a large central hole.	10
Figure (4)	Schematic alignment of the amino-acid sequences of yeast DNA topoisomerase II and E. coli DNA gyrase	11
Figure (5)	A molecular model for the catalytic reaction of topoisomerase II.	13
Figure (6)	Diagram of DNA topoisomerase II cleavage reaction. ATP binding cause the two DNA linked tyrosines to undergo a significant translocation away from each other which opens a gab large enough for the second duplex to pass through.	14
Figure (7)	Reactions catalysed by type II DNA topoisomerases.	15
Figure (8)	Two paths for the merging of a pair of converging replication forks	17
Figure (9)	 (A) top, a transcriptional ensemble R shown on a DNA segment whose ends are anchored to a certain cellular entity "E". (A) Bottom, formation of positive supercoils ahead of the polymerase and negative supercoils behind it. (B) The terminal ends of DNA "E" are combined. 	18
Figure (10)	DNA topoisomerase II poisons stabilize the cleavable complex in the topoisomerase II reaction by forming a drug- enzyme- DNA ternary complex which is required to trigger the lethal effect of topoisomerase II poisons.	28
Figures (11 & 12)	Measurement of the area percentage of positive nuclear topo IIα immunoexpression.	47
Figures (13-15)	Measurement of the optical density of positive nuclear topo IIα immunoexpression.	48 & 49

ú-List of Figures (continue)

Figure	Figure Caption	Pages
Fígure Number	·	
Figures	Histopathology and immunohistochemistry of the	59-61
(16-20)	control specimens	
Figures	Histopathology and immunohistochemistry of	62-65
(21-28)	pleomorphic adenoma.	
Figures	Histopathology and immunohistochemistry of Warthin's	66 & 67
(29-32)	tumour.	
Figure s	Histopathology and immunohistochemistry of malignant	68-70
(33-38)	pleomorphic adenoma.	
Figure s	Histopathology and immunohistochemistry of low grade	71 & 72
(39-42)	mucoepidermoid carcinoma.	
Figure s	Histopathology and immunohistochemistry of high grade	73-75
(43-47)	mucoepidermoid carcinoma.	
Figure s	Histopathology and immunohistochemistry of adenoid	76-82
(48-60)	cystic carcinoma.	
Figure s	Histopathology and immunohistochemistry of	83-85
(61-65)	polymorphous low grade adenocarcinoma.	
Figure s	Histopathology and immunohistochemistry of acinic cell	86 & 87
(66-68)	carcinoma.	
Figure s	Histopathology and immunohistochemistry of squamous	88 & 89
(69-72)	cell carcinoma.	
	Bar chart illustrating the difference in area percentage of	
Figure (73)	topo IIα positive cells in benign and malignant salivary	92
	gland tumours.	
	Bar chart illustrating the difference in immunostaining	
Figure (74)	intensity of topo IIα positive cells in benign and malignant	93
	salivary gland tumours.	
	Bar chart illustrating the difference in area percentage of	
Figure (75)	topo IIα positive cells among different histological	94
	patterns of adenoid cystic carcinoma.	
	Bar chart illustrating the difference in immunostaining	
Figure (76)	intensity of topo IIα positive cells among different	95
	histological patterns of adenoid cystic carcinoma.	
	Bar chart illustrating the difference in area percentage and	
Figure (77)	immunostaining intensity of topo IIα positive cells between	96
	pleomorphic adenoma and malignant pleomorphic	
	adenoma.	

Figure (78)	Bar chart illustrating the difference in area percentage and immunostaining intensity of topo IIα positive cells between low and high grade mucoepidermoid carcinoma.	97
--------------------	---	----

Introduction

DNA undergoes conformational and topological changes during many cellular processes such as replication and transcription (Liu, 1989). DNA topoisomerases are ubiquitous enzymes; they resolve the topological problems which arise during the various processes of DNA metabolism including transcription, recombination, replication and chromosome partitioning during cell division (Osheroff et al., 1991; Gasser et al., 1992).

Topoisomerases are classified into two major types, topoisomerase I and topoisomerase II (Tan et al., 1992). Because of their vital functions in cell physiology, they have become targets of numerous chemotherapeutic drugs (Chen and Liu, 1994; Wang, 1994).

Type I topoisomerases are involved in transcription, whereas type II topoisomerases play an important role in DNA replication and mitotic events. Two isoforms encoded by different genes were identified for type II, topo IIα and topo IIβ. They do not only differ in their biophysical and biochemical properties but are also differentially regulated during the cell cycle (Capranico et al., 1992; Jenkins et al., 1992; Hwang and Hwong, 1994).

Danks et al. (1993) and Ishida et al. (1994) found that mutation of the gene encoding for DNA topoisomerase IIα (topo IIα) may influence