“Effect Of Fucoxanthin On Head And Neck Squamous Cell Carcinoma Cell Line: In Vitro Study“

Thesis

Submitted to the Oral Pathology Department, Faculty of Dentistry, Ain Shams University for partial fulfillment of requirements of the Doctor Degree of Philosophy (PhD) in Oral and Maxillofacial Pathology

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Dedication

I would like to dedicate this work to all members of my family, specially my parents and my sisters for pushing me forward in every step of my life.

Also to my friends for their support and encouragement

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List of Abbreviations

Abbrev.	Full term
APC	**:** Adenomatous polyposis coli
BAX	**:** Bcl2 associated X
BMF	**:** Bcl2 modifying factor
BCR	**:** B cell receptor
CDK	**:** Cyclin dependent kinase
Cox2	**:** Cyclooxgenase 2
CXCR4	**:** Chemokine receptor 4
CYP1A1	**:** Cytochrome family 1 sub family A
EBV	**:** Epstein-Barr virus
EGFR	**:** Epidermal growth factor receptor
Fx	**:** Fucoxanthin
Fxol	**:** Fucoxanthinol
GADD	**:** Growth arrest and DNA damage inducible protein
GJIC	**:** Gap junction intracellular communication
GRB2	**:** Growth factor receptor-bound protein 2
HER2	**:** Human epidermal growth factor receptor 2
HNSCC	**:** Head and neck squamous cell carcinoma
HPV	**:** Human papilloma virus
HRK	**:** Harakiri gene
HUVE	**:** Human umbilical vein endothelial cells
ICAD	**:** Inhibitor of caspase activated DNA
IO	**:** Immuno-oncology
ITAM	**:** Immune receptor tyrosine based activation motif
KLF4	**:** Kruppel like factor 4

LAB : Linker for activation of B cells

LAT2 : Linker for activation of T cells

LCK : Lymphocyte specific protein tyrosine kinase

LFA1 : Lymphocyte function associated antigen 1

MMP : Matrix metalloproteinases

MiRNA : Micro ribonucleic acid

NK : Natural killer

NFAT : Nuclear factor of activated T cells

NOS : Nitric oxide synthase

NTAL : Non T cell activation linker

OD : Optical density

PARP : Poly ADP ribose polymerase

PIAS1 : Protein inhibitor of activated STAT1

PIM : Proviral integration site for Moloney murine leukemia virus-1

PUMA : P53 upregulated modulator of apoptosis

ROS : Reactive oxygen species

SLP76 : SH2 domain containing leukocyte protein of 76 KDA

SOCS1 : Suppressor of cytokine signaling 1

STAT : Signal transducer and activator of transcription

TAAs : Tumor associated antigens

TCR : T cell receptor

TNF : Tumor necrosis factor

TRAIL : Tumor necrosis factor related apoptosis inducer ligand

TRAPs : Transmembrane adaptor proteins

VEGF : Vascular endothelial growth factor

WHO : World health organization

XIAP : X linked inhibitor of apoptosis protein

ZAP70 : Zeta chain of T cell receptor associated protein kinase

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Introduction

Head and neck cancer constitutes the eighth leading cause of cancer-related deaths worldwide. Its incidence varies widely among different geographical areas: in North America and the European Union, head and neck cancer accounts for 3% to 4% of all cancer diagnosis, while in developing countries its incidence is higher due to smoke and drinking habits in combination with poor socioeconomic status ***(Siegel et al., 2014).***

The most common type of head and neck cancer is head and neck squamous cell carcinoma (HNSCC) which is a morbid, frequently lethal disease that affects the epithelial cell lining of the upper aero-digestive tract (UADT, i.e., the oral cavity, pharynx, and larynx) ***(Leemans et al., 2011)***. While HNSCC has been strongly associated with tobacco use and heavy alcohol consumption, over the past two decades, high-risk alpha human papillomaviruses (HR α-HPV) have been an important etiological factor for a subset of HNSCC arising from the oropharynx ***(Gillison et al., 2000)***.

Generally, HNSCC is treated by surgery and/or chemo-irradiation, which cause severe side-effects in more than 80 % of the advanced HNSCC patients ***(Conley, 2006)***. Unfortunately, other treatment options for HNSCC patients are limited to only one single targeted therapy available in the clinic and with variable benefit i.e. cetuximab, targeting epidermal growth factor receptor (EGFR) protein

(Huang et al., 2014). To expand personalized cancer care for HNSCC, novel targeted therapies are needed ***(Razzouk, 2014)***.

Fucoxanthin is a marine carotenoid pigment that is produced in brown seaweeds and some microalgae ***(Peng et al., 2011).*** In humans, dietary fucoxanthin is mainly metabolized to fucoxanthinol, the deacetylated form of fucoxanthin ***(Hashimoto et al., 2012)***, and which is considered to be an active form of fucoxanthin.

Fucoxanthin possesses a unique chemical structure that includes an allene bond, 5,6-monoepoxide and acetylated group. Fucoxanthin is one of the most abundant carotenoids and fucoxanthin-containing brown algae, such as wakame (*Undaria pinnatifida*) and kombu (*Laminaria japonica*), are commonly consumed in Asia ***(Satomi, 2017)***.

Studies have revealed several health benefits of fucoxanthin, including anti-inflammatory, anti-obesity, anti-diabetes, hepato-protective and cardiovascular-protective activities, in addition to its anticancer activity ***(Mikami and Hosokawa, 2013)***.

Fucoanthin and fucoxanthinol exert their anti-proliferative and cancer preventive influences via different molecular pathways involved in either cell cycle arrest, apoptosis, or metastasis ***(Kumar et al., 2013)***.

In addition, Fx has been shown to have anti-angiogenic potential using human umbilical vein endothelial (HUVEC) cells ***(Ganesan et al., 2013)***, thus, contributing to cancer prevention.

Immuno-oncology (I-O) is a young and growing field, the product of the many groundbreaking discoveries in immunology and cancer therapy in the last century. The novelty of this field is due to the historical controversy over whether or not the body's immune system could even respond to cancer at all. The idea was first proposed by William Coley in 1893, when he observed the remission of cancer in patients who had contracted acute bacterial infections ***(Coley, 1893)***; then followed by Paul Ehrlich in 1909, when he suggested that the immune system must have some role in preventing an outbreak of cancer in the body ***(Ichim, 2005)***. Unfortunately, their theories were opposed by those who did not see a plausible biological explanation, and who were convinced that cancer cells were indistinguishable from healthy cells to the body's lymphocytes. The breakthrough for modern I-O came in the 1960s, when it was accepted that lymphocytes are constantly eliminating precancerous cells throughout the body in a process called "immune-surveillance," and do in fact recognize them through tumor-associated antigens (TAAs) ***(Baldwin, 1966)***. This has gradually led to our understanding of cancer today.

The manipulation of immune checkpoints is the leading edge for the field of I-O. The activation of a lymphocyte, such as a cytotoxic T-cell, in adaptive immunity has been well-characterized and is known to involve the interaction between an antigen-presenting cell (APC) and the T-cell receptor (TCR), and associated coreceptors. Less explored are immune checkpoints, the body's natural defense against auto-immunity. This involves the binding of receptors on the lymphocyte

with associated ligands on the surface of the cancer cell that interfere with activation signals or induce apoptosis. Inhibition occurs almost as quickly as activation and balances the antigenic response of immune cells to avoid an attack on healthy cells. Tumor cells exploit this with an upregulation of inhibitory ligands, leaving them free to grow unchallenged by the immune system ***(Kamta et al., 2017)***.

The ability of immunotherapy to mediate cancer regression has been shown in a variety of malignancies. Head and neck squamous cell carcinoma (HNSCC) serves as a paradigm of immunosuppressive disease, as it is characterized by dysregulated cytokine profile, impaired function of immune effector cells and abnormalities in tumor-associated antigen (TAA) presentation ***(Ferris et al., 2006; Allen et al., 2007)***. In several countries, an increasing number of cases with oropharyngeal carcinoma (OPC) are attributed to human papillomavirus (HPV), especially type 16, rather than traditional risk factors such as tobacco and alcohol ***(Weinberger et al., 2006)***. However, regardless of the implicated cause, it is believed that the immune system plays a key role in tumorigenesis of HNSCC, as malignant cells evade immune surveillance using multiple mechanisms ***(Srivastava et al., 2013; Bauman and Ferris, 2014)***.

Signal transducer and activator of transcription (STAT) are a proteins family that mediate cellular response to cytokines, such as IL-6 and growth factors ***(Turkson et al., 1998)***.