BIOLOGICAL STUDY OF HIGH RISK HPV IN LARYNGEAL CARCINOMA

Thesis

Submitted for partial fulfillment of the doctoral degree in otorhinolaryngology

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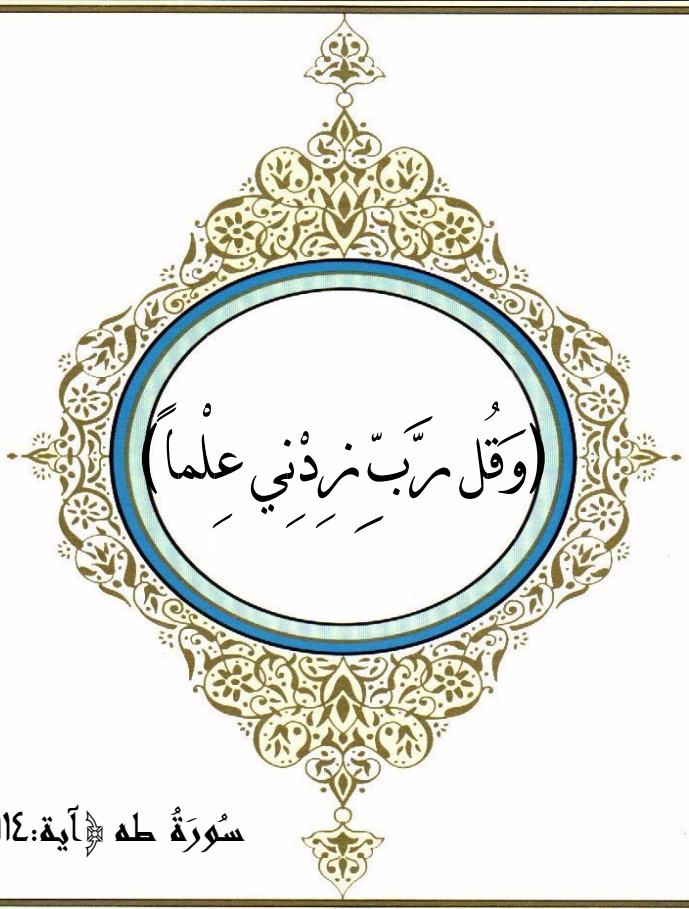

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LIST OF ABBREVIATIONS

AJCC	American Joint Committee on cancer
CAK	Cyclin activated kinase
CDK	Cyclin dependent Kinase
CIN	cervical intra-epithelial neoplasia
CKI	Cyclin dependent kinases associated inhibitors
E١, E٢ E٥, E٦, E٧	Early proteins
EGF	Epithelial growth factor
FGF١	fibroblast growth factor
FISH	fluorescence in situ hybridization
G٠	Non-dividing cells stage
G١	stage stands for "GAP ١"
G٢	stage stands for "GAP ٢"
Hdm-٢	Human double minute
HNSC	Head and Neck Squamous cell carcinoma
HPV	Human papillomavirus
IARC	International Agency for Research on Cancer
L١, L٢	late" proteins
LOH	loss of heterozygosity
M	stage stands for "mitosis"
Mdm-٢	Murine double minute
ORF	Open reading frames

TP٥٣	Protein product of tumor suppressor gene with molecular weight ٥٣ Kd
PCR	Polymerase chain reaction
R point	Restriction point
RB	Retinoblastoma
RT-PCR	reverse transcription-PCR
S	stage stands for "Synthesis"
SEER	Surveillance, Epidemiology & End Results
SV٤٠	Simian virus ٤٠
TGF alpha	transforming growth factor alpha
TSGs	tumor suppressor genes
WNT١	wingless/int-١

INTRODUCTION

Tobacco smoking and extensive alcohol drinking are known risk factors in the aetiology of head and neck squamous cell carcinoma (HNSC). Other known risk factors include environmental exposure to wool dust, wood dust and mineral fibers. However increasing number of (HNSC) in the absence of exposure to above risk factors suggests the presence of additional risk factors ***(Scully,*** ***٢٠٠٢)***.

In ***١٩٣٣, Shope and Hurst*** observed that infection with the cottontail rabbit Papillomavirus led to subsequent development of keratinous lesions, some of which progressed to invasive epithelial neoplasms. This observation led to the discovery of the first DNA virus that caused tumours in mammals. Substantial evidence now supports the role of HPV in the development of (premalignant) lesions of the vulva, penis, anus and uterine cervix. In addition, epidemiological and molecular data suggest that HPV may also promote head and neck carcinogenesis ***(Zur Hausen, ٢٠٠٢)***.

HPVs are small epitheliotrophic DNA viruses ≈ ٤٥-٥٥ nm in diameter with circular double strand DNA genome of ≈ ٨ Kb. Based on their capsid structure they belong to the family of papovaviridae. Nearly ١٢٠ HPV

genotypes have been identified, subdivided into benign and malignant or oncogenic subtypes. the benign subtypes, including HPV-٦ and -١١, are associated with mucosal warts and papillomatosis, such as occurs in the larynx, while others particularly HPV-١٦ and -١٨ are strongly associated with malignancy ***(Harriet et al., ٢٠٠٤)***.

Human papilloma virus share a common life cycle that can be divided into several steps. In the first step virus particles infect squamous epithelial cells. Initially, a latent infection is established, in which the tissue is clinically and histologically normal but the viral DNA is present in low copy numbers. The latent infection can persist in the absence of disease for the life of the host. The mechanism of activation of latent HPV infection is not known but two possibilities have been suggested; first minor irritation, wounding or exposure to ultraviolet (UV) light. Second, transient or local immunosuppression could permit viral activation ***(Benton et al., ١٩٩٢)***.

Activation of latency is accompanied by a marked increase in expression of HPV RNA in suprabasal cells, amplified synthesis of viral DNA in upper layers, and production of new virus particles in a subset of cells in the uppermost layers which is accompanied by increase level of viral proteins. Human papillomavirus DNA is organized into three regions. The early region contains the open

reading frames (ORFs) for all viral particles. These regions are denoted by an E preceding the number defining the individual ORF. E٦ and E٧ of the high risk HPV (HPV-١٦ and -١٨) can both immortalize and transform cell. The "late" regions L١ and L٢, code for the viral capsid proteins. These genes are expressed only late in viral life cycle after the viral DNA has been amplified to high levels. The third region of the genome, the upstream regulatory region (URR), does not code for viral proteins. It regulates viral gene expression and regulation ***(McMurray et al., ٢٠٠١)***.

The possible mechanisms associated with HPV-mediated carcinogenesis include both TP٥٣ mutation dependant and mutation independent pathway. The former mainly acts in upper aerodigestive tract tumors and the latter acts in cervical tumors. Mutation of the TP٥٣ tumor suppressor gene in upper aerodigestive tract is associated with alternations in the apoptotic regulatory bcl-٢ and bak genes, leading to down-regulation of programmed cell death and increased cell proliferation. HPV infection is also associated with increased tissue angiogenesis and activation of telomerase ***(Pillai and Nair, ٢٠٠٠)***.

Human papillomavirus can be detected by various methods. Southern blot is very specific and informative. However it has several limitations it is labor intensive, it can't be carried out on formalin-fixed and paraffin

embedded tissue. Hybridization in solution reduce amount of DNA required for assay. They are still limited to use of fresh or frozen material and provide HPV group identification, not specific HPV type. In situ hybridization uses labeled probes to detect HPV RNA or DNA in sections of tissue the major limitation is that ٥٠-١٠٠ copies of viral DNA per cell in multiple cells are required for detection. Polymerase chain reaction (PCR) is now the detection method of choice for most studies. It can be used to detect small numbers of HPV molecules, can be used with either fresh or archival materials can be established in a clinical laboratory, can be performed with either type specific primers or consensus primers that detect large numbers of different HPV types, and can detect a total of a few hundred molecules ***(Badaracco et al., ٢٠٠٠)***.

However its very sensitivity is the major limitation of PCR. Great care must be taken in handling specimens and preparing the reactions, to eliminate contamination that gives false-positive results. Although PCR can detect a small number of infected cells, it is not clear that this is biologically meaningful. Thus, some level of positive signal must be established to suggest a relationship between HPV positivity by PCR and disease. It is now recommended that semiquantitative RT-PCR be used to detect E٦ or E٧ transcripts, to assure that the virus is actively expressed in tumors ***(Matzow et al., ١٩٩٨)***.