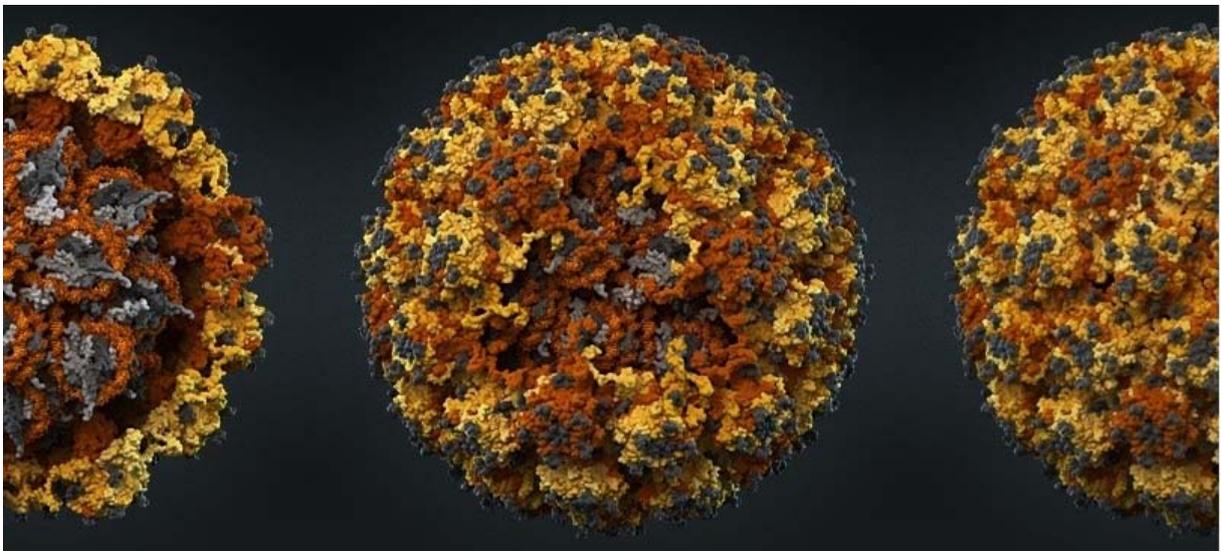# ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ
# ΤΜΗΜΑ ΒΙΟΧΗΜΕΙΑΣ & ΒΙΟΤΕΧΝΟΛΟΓΙΑΣ

## ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

## ΔΗΜΗΤΡΗΣ ΤΣΑΚΟΓΙΑΝΝΗΣ

## ΠΡΟΣΔΙΟΡΙΣΜΟΣ ΤΗΣ ΜΟΡΦΗΣ ΤΟΥ ΓΟΝΙΔΙΩΜΑΤΟΣ ΤΟΥ ΣΤΕΛΕΧΟΥΣ HPV-16, ΣΥΣΧΕΤΙΣΗ ΜΕ ΤΗΝ ΚΛΙΝΙΚΗ ΕΙΚΟΝΑ ΤΟΥ ΑΣΘΕΝΗ ΚΑΙ ΠΡΟΣΔΙΟΡΙΣΜΟΣ ΤΗΣ ΘΕΣΗΣ ΕΝΣΩΜΑΤΩΣΗΣ ΤΟΥ ΙΟΥ ΣΤΟ ΚΥΤΤΑΡΙΚΟ ΓΟΝΙΔΙΩΜΑ



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## Abstract

Human Papillomaviruses (HPVs) are non enveloped, epitheliotropic, double stranded, circular DNA viruses that infect cutaneous and/or mucosal epithelia. Over 150 different HPV types have been characterized, and approximately 40 of them are detected in the cervical epithelia and are classified as members of the genus Alphapapillomavirus. Epidemiological studies have revealed that persistent infection with high-risk human papillomavirus is the main risk factor for the development of high grade precancerous lesions and cervical carcinoma, with HPV16 and HPV18 types being the most frequently identified HPV types in invasive cervical cancer cases worldwide. Cancer progression is associated with persistent high-risk HPV infection and with deregulated expression of viral oncogenes E6 and E7. The E6 and E7 oncoproteins mediate mitogenic and antiapoptotic stimuli by interacting with numerous regulatory proteins of the host cell that control the cell cycle. As a consequence, HPV infection causes excessive cell proliferation, deficient DNA repair, and the accumulation of genetic damages in the infected cell.

Persistent infection with high-risk HPV types is associated with an increasing risk of genome integration into the host cell chromosome and progression to cancer. Viral integration deregulates the expression of the E6 and E7 proteins in the high-risk HPVs types. As a consequence, the virus stimulates cell cycle entry, providing selective growth advantage to the infected cells. In addition, the extensive proliferation of the infected epithelial cells is directly responsible for the accumulation of genetic errors, and genome destabilization that result in cancer development.

The present thesis, focused on the molecular analysis of HPV16 DNA. Moreover, the HPV16 DNA physical state and the HPV16 integration sites into the cellular chromosome were determined. The study was carried out in HPV16 positive cervical samples derived from Greek population, that were diagnosed as high grade, low grade squamous intraepithelial lesions and cervical cancer cases. Nucleotide and phylogenetic analysis of HPV16 DNA, showed that the prototype stain and the European variant are both prevalent in Greek population, while non European variants are circulating among Greek women. To the best of our knowledge, novel sequence

variations were reported for the first time within E1, E2, E4, E6 and E7 genes, as well as duplications and intratypic recombination events among distinct HPV16 variants were also reported. However, molecular evolutionary analysis proposed that different genes at the early region of HPV16 genome are dominated by different selective pressure and that might be associated with the essential role of viral proteins in the productive viral life cycle.

In addition, the present thesis studied the physical state of HPV16 DNA, considering that the key importance for the progression of cervical intraepithelial neoplasias (CIN) to invasive cervical cancer is the integration of HPV into the host genome. The physical state of viral genome was determined, taking into consideration the most frequently disrupted sites of HPV16 DNA, revealing that the viral integration is an early event in the viral life cycle. Finally, according to integration sites analysis, rearrangements within HPV16 genome were determined. In particular, a model of looping was proposed by which viral - host DNA concatatemers - mediate replication and recombination resulting to rearrangements in inverted orientation. According to this hypothesis, after HPV16 integration into the host chromososome, the virus promotes genome destabilization leading to cancer development. In conclusion, the extensive mapping analysis of disruption sites of E1 and E2 genes, in combination with the nucleotide analysis of E1, E2 and E6 genes, and the detection of HPV16 genome rearrangements, is a prerequisite in order to further understand and analyze the physical state of HPV16 DNA form in cancer progression.