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**Title of Thesis: Cellular and Viral Gene Interaction and their  
Regulation during Development of Esophageal Cancer**



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ABSTRACT

Esophageal squamous cell carcinoma (ESCC) is one of the most common cancers in the world with extremely poor prognosis due to late presentation and rapid progression. ESCC shows a great variation in geographic distributions and the incidence rates are remarkably higher in distinct areas. The wide geographical variation in the incidence reflects the strong influence of environmental factors. This cancer is also a major health problem in India; particularly in Kashmir valley and North-East India and is associated with distinct food and drinking habits such as drinking of hot salted tea which contains carcinogenic compounds like nitrosamines. Though recent reports have documented alterations of some oncogenes and tumor suppressor genes, the exact molecular and genetic basis of esophageal carcinogenesis still remains poorly understood.

Several studies have demonstrated infection of human papillomaviruses (HPVs) in esophageal cancer world over. These studies indicate an oncogenic role of HR-HPV types during esophageal carcinogenesis. Since HPV does not have its own transcriptional machinery, the expression of its two transforming oncogenes, E6 and E7 depends primarily on availability of host cell transcription factors, particularly the Activator Protein -1 (AP-1) and Nuclear factor kappa B (NF-kB). Currently there is no study that defines the role of AP-1 and NF-kB in HPV induced esophageal carcinogenesis.

**The present study encompasses the following findings:**

- The overall prevalence of HPV infection in esophageal cancer was found to be 19%. All the cancer cases were exclusively infected with high-risk HPV (HR-HPV) type 16 and no other HPV infection could be detected. Also, none of the normal adjacent tissue biopsies showed HPV infection.
- HPV positivity from Kashmir valley was found to be 19% (14/75) and 21% (8/38) from North-east India.
- Association of HPV infection with various clinico-pathological and demographic characteristics revealed significant association of HPV infection in patients with smoking history or having any grade (I, II or III) of dysphagia. A higher occurrence of HPV infection was also detected in advanced stage of cancer both clinically as well as histologically.
- Consumption of bicarbonate-containing hot salted tea commonly consumed by Kashmiri people appears to be a high risk factor for esophageal carcinogenesis.
- We demonstrate for the first time the constitutive activation and high DNA binding activity of AP-1 and differential expression pattern of its members between HPV positive and HPV negative esophageal tumor tissues.
- In the absence of HPV infection, AP-1 activity was composed of cFos, JunB, and Fra-1, while HPV infection was associated with elevated activity of AP-1 which was found to have an additional participation of JunD but absence of Fra-1.
- Unlike in other cancers, a high expression of its negative regulator Fra-1 was observed in HPV16 negative carcinomas.
- We observed a high DNA binding activity of NF-kB in esophageal cancer lesions compared to normal adjacent tissues.

- In the absence of HPV infection, NF- $\kappa$ B was composed of p50/p50 homodimers, while in HPV infected cases NF- $\kappa$ B was mainly composed of p50/cRel along with p65 heterodimers.
- The high level expression of NF- $\kappa$ B family proteins (p50, p65 and cRel) was observed in tumor tissues which was independent of HPV infection, while a very low or negligible expression of these proteins were observed in normal adjacent tissues.
- The high DNA binding activity and expression pattern of AP-1 and NF- $\kappa$ B was associated with esophageal cancer lesions and was independent of any histopathological grade of the disease.
- The gene expression of esophageal cancer on a microarray platform using customized Human cancer Ocichip revealed a total of 124 genes that were differentially expressed; of these 96 genes were upregulated and 28 genes were downregulated during esophageal carcinogenesis.

The present investigation provides evidence that the two most essential transcription factors AP-1 and NF- $\kappa$ B are constitutively activated in esophageal cancer and that they demonstrate a dichotomy and differential activation with respect to human papillomavirus infection and discovers specific impact of this virus on the composition of AP-1 and NF- $\kappa$ B complex. This in conjunction with other factors can contribute significantly to the development and progression of esophageal carcinogenesis and final outcome of the disease.

The current study also suggests that some of the novel genes that have emerged out of gene expression profiling are controlled by AP-1 and NF- $\kappa$ B transcription factors appears to play an important role in esophageal cancer and can act as biomarkers for early detection of esophageal cancer and their effective management. However, further studies are needed to evaluate and validate these gene functions before being considered as potential molecular markers for early detection and for treatment response specific to esophageal cancer.

There is a considerable interest in the prospects of development both prophylactic as well as therapeutic HPV vaccines to eliminate cervical cancer. It would be excellent if the rates of esophageal cancer and perhaps other epithelial cancers also drop and it would be the most convincing proof that HPVs are also involved in the development of esophageal cancer.