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Title of the thesis: **"Study the Associative Role of Alteration(s) in Cell Cycle Controller EZH2, Tumour Suppressor RUNX3 and DNA Repair Gene MGMT in Esophageal Cancer"**

Runt related transcription factor3 (*RUNX3*) a known tumor suppressor gene (TSG) that exploits the TGF-β pathway dependent apoptosis. Previous studies have indicated that RUNX3 is inactivated in various cancers especially gastrointestinal cancers. Its expression is usually brought down about by hypermethylation of CpG islands of *RUNX3* promoter, and also it has been seen that the overexpression of enhancer of zeste homolog 2 (*EZH2*) downregulates *RUNX3* in gastric cancer among the various other. However, RUNX3 role in esophageal cancer in North Indian patients and its association with the EZH2 is unknown. O-6-Methylguanine-DNA Methyltransferase (MGMT), DNA repair gene has been found to be involved with the pathogenesis of the esophageal cancer. DNA hypermethylation and other factors have been suggested to downregulate the MGMT. Therefore, we contemplated to study the expressions of *RUNX3*, *EZH2* and *MGMT* in tumor tissues along with adjacent normal mucosa and tried to figure out the relationship between the RUNX3 and EZH2. q-PCR was used to study the mRNA expression levels of RUNX3, MGMT and EZH2, immunoblotting and immunohistochemistry gave us the picture about the protein expressions and subcellular locations of both the proteins. Methylation specific-PCR was employed to assess the level of DNA methylation at the CpG islands of the RUNX3 & MGMT promoter region. *EZH2*, *MGMT* & *RUNX3* expression was lately correlated with the Clinicopathological parameters and the survival analysis was done. Squamous cell carcinoma and Adenocarcinoma accounted for 89.6% and 11.4% respectively of the included 80 patient cases of the North Indian origin. 8.7% of the tumor samples had acquired malignancy. 56.9%

cases fell in the advanced stages of cancer and 43.1% had cancer in stage I or stage II. In 47/80 patient samples RUNX3 mRNA was found to be upregulated in the tumor tissue when compared to the normal mucosa ($p < 0.017$). DNA hypermethylation at the promoter region of the *RUNX3* was seen in 28 patients with downregulated RUNX3 expression ($p<0.001$). We didn't get any significant upregulation in the EZH2 mRNA expression however, RUNX3 and EZH2 were found to be significantly correlating each other ($p < 0.03$). MGMT mRNA expression was found to be downregulated in 65% cases (52/80). The expression of MGMT at the protein level was absent in 65% (52/80) cases. 52 cases had low or no expression of the protein whereas out of 28 remaining cases 11.25% (09/80) cases had highly expressing MGMT protein. Absence of MGMT protein coincided with the methylated cases in 82% (37/45), whereas in 08 cases out of the 45 methylated, MGMT protein was present. Therefore, a very strong correlation among the MGMT promoter methylation and the MGMT protein expression was seen ($p<0.0002$). The aggressive esophageal cancer patients had MGMT protein loss significantly correlating the hypermethylation at the promoter region with stage III and stage IV ($P\leq 0.001$), MGMT loss and hypermethylated in stage III & IV (51%) vs. stage I & II (40%). Loss of MGMT protein was very frequent in the incidence of esophageal cancer from North Indian patients and hypermethylation of the promoter region of MGMT was significantly associated in its downregulation. Therefore, it can be concluded that RUNX3, EZH2 and MGMT expression in esophageal cancer patients from north Indian population does embarks more studies to regain the firmness behind the scenario of their functioning.