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Title of the thesis: "Study of certain Tumor suppressor gene and oncogenic association in the modulation of cell cycle regulators in different Gastrointestinal cell lines".
Keywords: PARK2/Parkin, Colorectal cancer, P53, cell cycle, apoptosis, Gene Expression

Cancer, a second leading cause of mortality worldwide is a serious problem affecting human health of all societies. PARK2 (PARKIN) is an E3 ubiquitin ligase and it has been implicated in protein turnover, stress response, mitochondria homeostasis, xenophagy, metabolism, and numerous other cellular processes amendable cell growth and endurance. P53 acts as a tumor suppressor protein that helps to regulate normal cell growth, multiplication by holding the cell cycle at the G1/S regulation point on DNA damage recognition. Interestingly, Parkin is a transcriptional target gene of P53 in controlling glycolysis and plays crucial role in Warburg effect which provides a new axis to carcinogenesis; unexplored in case of colorectal cancer so far. This thesis has highlighted the possible synergistic molecular associations between Parkin and P53 protein expression and colorectal tumorigenicity. A programmed cell death mechanism has also been investigated through overexpression of Parkin and P53 in colorectal cancer cells expressing genetically different patterns of the same genes at basal level; HCT116 expressing wild type Parkin and P53, HCT116P53-/- expressing Parkin but devoid of P53 completely. Another metastatic derivative of colorectal cancer cell line SW620 has also been used in this study.

The basal level expression of Parkin assessed through western blot in the three selected cell lines HCT116, HCT116P53-/- and SW620 was found to be highest in HCT116 cells as compared to HCT116P53-/- and SW620. The expressional level of *Parkin* showed a considerable increase upon WT-P53 transfection, illustrating transcriptional regulation by P53. We did not find any repressive effect of *Parkin* on P53 protein, while on silencing P53 through p53 targeted shRNA, the expressional level of *Parkin* was decreased. There was no effect/difference observed in the expression of Parkin in transfected HCT116P53-/- cells with mutant P53. Cells transfected with wild type Parkin and P53 expressing vectors showed a decline in the proliferation rate in HCT116 to 84 percent and 71 percent respectively after 48 h and 96 hours of transfection. However, HCT116P53-/- cells, which lack endogenous P53 expression, showed 84 percent and 75 percent viability. The down-regulation of Parkin in the

colorectal cancer cells was found to promote cells to overcome G0/G1 phase of cell cycle. The tendency to overcome G0/G1 phase was found to be maximum in Parkin shRNA expressing HCT116P53-/- cells than the HCT116 cells which has wild type P53 expressional status. We also found that in HCT116 cells, the ectopic expression of Parkin targeted shRNA caused fragmentation of the nucleus and the number of colonies counted in Parkin shRNA transfected HCT116P53-/- cells were found to be maximum reaching nearly 300 as compared to the control cells where the number of colonies were about 115 in number. Parkin shRNA expressing HCT-116P53-/- cells demonstrated near to a complete closure of the scratch by 24 hrs compared to control cells. Nearly 80 percent of the total area was covered by the cells which confirmed the highly invasive nature of cells upon Parkin knock down and absence of P53 protein. P53 and Parkin knock down HCT116 cells showed lower migration rates where P53 shRNA and Parkin shRNA expressing cells covered around 75 percent and 63 percent of area respectively after 24 hours. Ectopically expressed wild type Parkin/P53 caused apoptosis in all the three cell lines (HCT116, SW620 and HCT116P53-/-) with different Parkin and P53 expressional status. Although, the highest apoptotic population of cells was observed in HCT116 cells expressing wild type P53 vector plasmid; demonstrating 15.4 percent cell death. The western blot data demonstrated a relative increase in the expression of *Parkin* in HCT116 cells overexpressed with wild type P53. Most importantly, the overexpression of both wild type *Parkin* and P53 in HCT116 cells have resulted in the expressional alterations of two crucial apoptosis related proteins; the proapoptotic Bax and the anti-apoptotic Bcl2. The expressional results also show that the overexpression of P53 induces more apoptosis in HCT116 (having WT-P53) cells than by overexpressing with *Parkin* alone.

Through a comprehensive analysis of *Parkin* in colorectal cancer cell lines through this work, we have presented several lines of evidence that indicate an important role of cell cycle regulators like Cycin E, Bax, Bcl2, P53 in *Parkin* regulation. In our present study, we have found that PARK2 acts as a significant regulator of programmed cell death and have demonstrated to promote P53 mediated cell death. The relative expression of both the apoptotic related proteins were found to change upon Parkin overexpression. Since, there is an increase in the level of Bax expression and Bcl2 down-regulation; we can say that cells are undergoing apoptotic pathway; highlighting Parkin to be a potent tumor suppressor gene. Thus, as a direct P53 target, *Parkin* in all likelihood, contributes to the functions of P53 in tumor suppression throughout the regulation of cell cycle, apoptosis, migration and proliferative behaviour of the cells.