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Title of Thesis: **Molecular Analysis of Parkin Gene in Different Gynaecological Cancers**

FINDINGS OF THE STUDY

The current study is based on the evaluation of *Parkin* gene alterations in gynaecological cancers. In this study, the involvement of *Parkin* gene in two gynaecological cancers such as cervical and ovarian cancer were evaluated in terms of mutational analysis using loss of heterozygosity and PCR-single stranded conformational polymorphism and *Parkin* expression by RT-PCR. Out of 105 cervical tumors and 102 ovarian tumors examined, 59 (56%) samples cervical cancer specimens and 64 (62%) ovarian samples showed LOH in at least one locus in the region examined. The highest rate of LOH was observed at intragenic markers D6S305 and D6S1599 which is located towards centromeric end between exons 7 and 8 where as the other is located in the 5' end of the *Parkin* gene, between exons 2 and 3 respectively. Analysis of *Parkin* gene expression found transcript levels to be reduced or absent in >35% and 45% of the cervical and ovarian specimens examined respectively. No or reduced *Parkin* gene expression was observed in those cervical specimens that belonged to grade III and stage III-IV, where as in case of ovarian tumors, the reduced expression was found in the specimens of stage III. It suggests that *Parkin* gene deletion might be associated with a histological subtype and its downregulation might have a unique association with the disease progression. Out of 105 cervical tumor samples, 24 (23%) samples and out of 102 ovarian tumor samples, 30 (29%) samples exhibited both LOH and reduced *Parkin* gene expression. Six of the cervical and 10 of ovarian tumor specimens demonstrated either reduced *Parkin* gene expression or no expression in those samples which showed common region of loss in both the intragenic markers D6S305 and D6S1599 which involves *Parkin* exons 2-10. It suggests that the expression of *Parkin* transcript might have some relation with genomic deletions. In addition, 12 out of 105 (11%) cervical and 16 out of 102 (16%) ovarian tumor samples, which retained heterozygosity in our LOH analysis defined by the intragenic markers D6S305 and D6S1599, exhibited a reduction in *Parkin* gene expression. It indicates that a mechanism other than deletion may account for the reduction in the levels of *Parkin* in these tumors.

In this study, we have found the novel substitution and frameshift mutations in *Parkin* exons 4 and 8 in cervical carcinoma. One sample has shown alteration in the

sequence at two different codons of *Parkin* exon 4 in which AA was replaced by GG at codon 155 as a result of which the codon sequence changes from CAA to CGG resulting in the change of amino acid from Glutamine (Q) to Arginine (R). In the same sample at codon 165, insertion of C results in the change of codons from CAG to CAC and the resulting amino changes from Gluatamine (Q) to Histidine (H) and hence the whole amino acid sequence changes due to this insertion. One sample has shown insertion of A in the same exon at codon 177 leads to the change of codon TTG to TTA and hence the codon 178 changes, resulting in the change of amino aid Threonine (T) to Aspartic Acid (D) and the next amino acid also changes from Glutamine (Q) to Proline (P). 3 samples have shown an insertion of C at codon 157 of *Parkin* exon 4 which results in the change of codon from TGC to TCG as a result of which the resulting amino acid at codon 158 changes from Glutamine (Q) to Alanine (A) and hence the whole sequences of amino acid changes. 3 samples have shown a substitution mutation at codon 310 of *Parkin* exon 8, replacing G by A as a result of which the codon changes from GAG to GAA resulting in no of amino acid i.e.Glutamic Acid (E). Most of the *Parkin* mutations were observed in the specimens belonging to grade II and stage IV. We have also reported the novel frameshift mutations in *Parkin* exon 4 in ovarian carcinoma. 4 samples have shown an insertion of C at codon 157 of *Parkin* exon 4 which results in the change of codon from TGC to TCG as a result of which the resulting amino acid at codon 158 changes from Glutamine (Q) to Alanine (A) and hence the whole sequences of amino acid changes. In the same sample, At codon 177, insertion of A leads to the change of codon TTG to TTA and hence the codon 178 changes, resulting in the change in amino acid sequences. One sample depicted an insertion of A at codon 177, resulting in the change of amino acid Threonine (T) to Aspartic Acid (D) and the next amino acid also changes from Glutamine (Q) to Proline (P). This study reveals a very interesting fact that the reduced *Parkin* mRNA expression is associated with the deletion in *Parkin* gene locus. All the samples that have shown no or reduced *Parkin* expression were also found to be a target of intragenic mutations at different *Parkin* gene loci in both cervical as well as ovarian carcinoma.

This finding suggests that *Parkin* downregulation has some close association with the *Parkin* mutation in the progression of these gynecological cancers. We further wish to propose that it would require some additional work, especially on the front of mechanism of action to exactly underline the role of this gene in human malignancies as a member of TSG family.