Molecular Studies on *Parkin Gene* among Breast Cancer Patients

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Abstract

Breast cancer is one of the most common causes of cancer related death in women all over the world. It is a most heterogeneous disease thus the classical prognostic factors are limited and often fail to predict the clinical course of the disease. Therefore, developing improved prognostic tools that define the specific subtypes of breast cancer based on genetic and epigenetic parameters has a potential to individualized treatment options and efficacy.

Parkin is a potential tumor suppressor gene hence its down regulation might be a contributing factor of breast cancer by affecting pathways/processes in the body. Therefore, it was of great interest to assess other tumour suppressor genes and their association with *Parkin* to address the missing association/networks in breast cancer. This study was aimed at defining gene expression changes of tumor suppressor gene (TSG) *Parkin*, along with other TSGs; *BRCA1*, *BRCA2* and *p53* to evaluate their prognostic relevance in breast carcinoma patients. Histological analyses were carried out from formalin-fixed, paraffin-embedded primary tumor specimens and *Parkin*, *BRCA1*, *BRCA2* and *p53* statuses were systematically analyzed by immunohistochemistry. We found reduced/loss of *Parkin* expression in breast cancer tissues and cell lines, which was significantly correlated with more aggressive and potentially metastatic triple negative breast cancers. Data here for the first time provides evidence that

aberrant promoter methylation of *Parkin* is a major event in it loss/reduced expression. The correlation of *Parkin* expression with its methylation was validated by RT-PCR and MS-PCR in MCF-7, MDA-MB-231, MDA-MB-468 breast cancer cell lines. Remarkably, *Parkin* expression and its promoter methylation both were correlated with the overall survival of the patients with breast carcinoma. Following epigenetic study we also analysed the mutation of *Parkin* gene where we found two novel variant of *Parkin.* However, no correlation was observed between the *Parkin* mutation and protein expression as well as with the clinical variables. While analyzing the expressional association we found a strong positive relationship between the *Parkin* and *BRCA1* expression while a negative association was observed between the *Parkin* and *p53* expression. The study showed no significant correlation between the expressional patterns of *Parkin* and *BRCA2*.

The overall aim of this thesis is to increase the understanding of the biological role of *Parkin* expression and its correlation with other tumor suppressor genes along with its prognostic role in breast cancer. For this we also analyzed associations between gene expression in breast cancer and patient survival using the log rank test. In survival analysis, we found that the simultaneous loss of *Parkin* with *BRCA1* or *BRCA2* and positive expression of *p53* characterizes poor survival of the patients. The independent prognostic values of the clinical parameters as well as genes ranking the highest in univariate analysis were assessed with Cox multivariate regression analysis. Our analysis demonstrated that *Parkin* and *BRCA1* both are independent prognostic markers for the survival of a clinically aggressive breast carcinoma. Given the importance of Parkin and its association with other classical genes and clinical parameters make it a likely candidate of future investigation. Expansion of the genetic approaches and association studies detailed herein may enable us to find markers of susceptibility, development/ progression of breast cancer.