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Topic of Research: Molecular Analysis of FOXN3 and MEN1 genes in Indian Breast Cancer Patients.

FINDINGS

FOXN3 and MEN1 are reported to be involved in various bioprocesses and their role in different types of cancer is well documented. However, there is limited literature available addressing their involvement in breast cancer, we in the current study focused upon evaluating the role of FOXN3 and MEN1 genes in Indian breast cancer patients.

Our mRNA expression analysis revealed that FOXN3 and MEN1 mRNA were upregulated in the breast tumor samples when compared with adjacent normal breast tissue. Overall, 61.26 % and 63.38% of cases exhibited the overexpression of FOXN3 and MEN1 mRNA respectively in the breast tumor samples. When we investigated their association with clinicopathological parameters of the patients, we found that the elevated mRNA expression of both the gene was significantly correlated with positive lymph node status of the patients thereby indicating their role in invasion and metastasis. Also, we found that higher expression of FOXN3 mRNA was significantly associated with the post-menopausal status of the patients. Further, elevated MEN1 mRNA expression also showed strong correlation with positive estrogen receptor status and age of the patients.

Immunohistochemistry and Western blotting were performed to evaluate the FOXN3 and MEN1 protein expression in breast cancer cases. We in our study report overexpression of both FOXN3 and MEN1 protein in the breast tumor samples than adjacent normal breast tissue samples. 59.15% cases showed the higher expression of FOXN3 protein while 60.56% cases exhibited elevated expression of MEN1 protein in the breast cancer cases. When the findings were correlated with the clinicopathological parameters of the patients, we found that elevated FOXN3 protein expression was significantly correlated with the positive lymph node status and advanced clinical stages of the patients. Further when MEN1 protein expression compared with the clinicopathological parameters of the patients, a strong correlation with positive estrogen status of the patients was observed. Our findings revealed

that FOXN3 protein expression was significantly higher in stage III and IV of breast cancer suggesting that elevated expression of FOXN3 can have a significant role in prognosis of the disease.

Further to evaluate any epigenetic or genetic alterations in the promoter or coding region of both the genes; Methylation specific PCR and Sangers sequencing were performed respectively. We found 73, 19 and 50 out of 142 cases to be unmethylated, methylated and unaltered at the FOXN3 promoter region respectively. Similarly, the promoter methylation analysis for MEN1 promoter region revealed 76, 29 and 37 out of 142 cases to be unmethylated, methylated and unaltered respectively. We found that the elevated expression of FOXN3 and MEN1 was significantly correlated with the unmethylation at their promoter regions. Further, we performed a statistical analysis to evaluate the statistical correlation between promoter methylation status and gene expression. Our data do not reveal any significant correlation between FOXN3 promoter methylation status and clinicopathological parameters of the patients. However, a significant correlation was observed between MEN1 promoter methylation statius and advanced clinical stages of the patients where the majority of the cases in stage III and IV exhibited the promoter unmethylation. We also found that the age of the patients at the time of menopause showed significant correlation where 30 out of 45 early menopausal cases had MEN1 promoter unmethylation. The mutational analysis for FOXN3 and MEN1 gene do not reveal any mutation in Indian breast cancer patients.

Our findings using oncoDB revealed similar results and we report that both FOXN3 and MEN1 are associated with poor survival in breast cancer patients. Moreover, we evaluated the interaction of both the genes with immune cell infiltration ising TISIDB. FOXN3 and MEN1 were found to be significantly correlated with various tumor infiltrating immune cells including Tregs that have immuno-suppressive roles. Hereby, we can conclude that FOXN3 and MEN1 can have probable role in tumor associated immunoediting and can also be involved in immonoresistance. We performed pathway enrichment and Gene ontology analysis to find the association of the two genes in various biological processes. The enrichment network analysis revealed the inter-connection of FOXN3 and MEN1 with “Negative regulation of nucleic acid templated transcription”.

In conclusion, our findings revealed that both FOXN3 and MEN1 are overexpressed in Indian breast cancer patients. However, when we correlated the expression of the two genes, we did not find any significant correlation in their expression.