ABSTRACT

Background: Early diagnosis of cancer is imperative to stop the spread of the disease and for the long-term survival of the patients. Focus on detecting symptomatic patients as early as possible gives the best opportunity for successful treatment. But the failure to detect the rare cancer stem cells (CSCs) population that uniquely initiates and sustains the disease poses a major challenge to early cancer detection. Recent reports indicate the contribution of exosomes, secreted by CSCs to orchestrate the formation and maintenance of tumors promoting and metastasizing environment.

Methods: Breast CSCs (bCSCs) were purified from breast cancer cell lines and human breast tumor tissues following which CSC-derived exosomes (CDEs) were isolated. CDEs were isolated from the tumor blood samples and characterized through DLS, AFM, and western blot analysis. CDE-FOPX3 was detected through flow cytometry, western blot, and mass spectrometry analysis.

Results: We observed a higher level of FOXP3 expression in MDA-MB-468 CSC-derived exosomes (CDEs) as compared to exosomes isolated from non-malignant MCF10A derived stem cells. Interestingly, CDE isolated from breast cancer patients also showed a comparable FOXP3 expression. A strong positive correlation between CSC percentage and CDE-FOXP3 expression in tumor patients was also observed. Furthermore, we report significant elevation of CDE-FOXP3 in peripheral blood of 14 clinically diagnosed breast cancer patients compared to 19 healthy individuals as a non-maligned, open-label, case-control study. The clearly detectable non-overlapping ranges of CDE-FOXP3 in breast cancer patients and healthy cohorts highlight the potential of CDE-FOXP3 to be a low cost blood-based detection marker eligible for a phase-I clinical study.

Conclusions: Detection of CDE-FOXP3 in the peripheral blood of breast cancer patients will allow us to detect CSC in a non-invasive method. Its expression is distinguishable from healthy butters due to clearly delineable ranges and remain unchanged even during chemotherapy, suggesting future prospects of the same in an early diagnostic marker. Collectively our data demonstrate that detecting CDE-FOXP3 can become a quick non-invasive way to diagnose and screen early-stage breast cancer in a cost-effective manner.

RESULTS

Figure 1: A) Flow cytometry analysis showing the expression of FOXP3 in MDA-MB-468 CSCs as compared to MDA-MB-468 cells. B) Positive correlation was observed between FOXP3 and SOX2 in CSCs as compared to MDA-MB-468 cells.

Figure 2: A) TCGA data mining showing high expression of FOXP3 in tumor tissue and expression pattern of FOXP3 based on patient’s race. B) Schematic diagram describing the workflow. C) Relative mRNA expressions of CSC signature gene and FOXP3. D) Positive correlation between mRNA expression pattern of SOX2 and FOXP3 in CSCs isolated from breast tumor tissues.

Figure 3: Correlation of FOXP3 with CSC signature gene SOX2 was found to be non-significant in mammary stem cell and differentiated mammary cell.

Figure 4: Expression of FOXP3 protein was observed to be higher in MDA-MB-468 CSCs and CDEs as compared to non-malignant MCF10A and stem cell-derived exosomes.

Figure 5: CDE-FOXP3 was detected in the peripheral blood of breast cancer patients.

CONCLUSIONS

➢ FOXP3 is overexpressed in breast CSCs as compared to mammary stem cells and differentiated mammary cells.

➢ FOXP3 was found to be positively correlated to stemness marker SOX2 only in CSCs.

➢ FOXP3 was found to be secreted by breast CSCs and mammary stem cells via exosomes.

➢ The concentration of FOXP3 secreted in CSC-derived exosomes where higher as compared to mammary stem cell-derived exosomes.

➢ This exosomal-FOXP3 was also detected in the peripheral blood of breast cancer patients and its range was found to be non-overlapping to the range observed in healthy persons.

FUTURE PERSPECTIVES

➢ This preliminary study can be expanded to a larger cohort of patients to further validate it.

➢ As FOXP3 is strongly correlated to CSC signature gene SOX2, it can serve as a surrogate marker to detect the presence of CSCs which are responsible for tumor initiation.

➢ If validated in a larger cohort, exosomal-FOXP3 can be utilized in the diagnosis of early stage breast carcinoma as it can be easily detected via a non-invasive liquid biopsy test.

REFERENCES


ACKNOWLEDGEMENTS

The authors would like to thank Council of Scientific and Industrial Research (CSIR), Department of Biotechnology (DBT), Government of India for funding the present study. The authors would also like to thank ICMR-NBRI, Kolkata for providing the necessary facilities to carry out the research. The authors would also like to thank Dr Debolata Sengupta for assisting us during abstract writing.