Functional profiling of the Stress-Induced Phosphoprotein 1 (STIP1) interactome in breast cancer using high-throughput IP-MS/MS

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Breast cancer is the most common malignancy worldwide, characterized by significant heterogeneity and various functional phenotypes, with a complex array of histological and molecular features. Proteomics technologies are essential for assessing protein profile changes, discovering cancer biomarkers, detecting genetic alterations, and mapping functional pathways, thereby differentiating breast cancer subtypes. A key mechanism for regulating the proteome balance in cells is proteostasis, which involves a complex network of pathways controlling the synthesis, folding, maintenance, trafficking, and degradation of proteins. Chaperones and their regulatory cofactors play a major role in coordinating these processes. Stress-induced phosphoprotein 1 (STIP1) is particularly important as it helps to coordinate the functions of heat shock proteins HSP70/90 in protein folding, preventing aggregation and misfolding. Dysregulation of proteostasis is associated with various pathological conditions, including cancer, with many HSPs and STIP1 often being overexpressed. In this study, label-free quantification mass spectrometry, immunoprecipitation, and bioinformatics approaches were employed to analyze the proteome profiling of breast cancer subtypes compared to paired controls: luminal A, luminal B, HER2-enriched, and triple-negative. A total of 484 differentially expressed proteins were identified among the groups. We observed increased levels of STIP1 across all breast cancer subtypes. In silico analyses also revealed STIP1 overexpression at both the mRNA and protein levels in breast tumors. Furthermore, higher STIP1 expression in triple-negative cell lines was confirmed by western blotting. Based on these data and our previous proteomic studies, we applied immunoprecipitation coupled with mass spectrometry (IP-MS) to identify STIP1 binding partners in the MDA-MB-231 triple-negative breast cancer cell line. This analysis employed TRITON-X 100 lysis buffer and magnetic beads conjugated with proteins A/G. The efficiency of precipitation was assessed by SDS-PAGE and western blotting, with the MDA-MB-231 full proteome serving as the control. The IP-MS analysis identified a total of 132 high-confidence STIP1 interaction partners ($p<0.01$). The STIP1 interactome was predominantly composed of HSPs, chaperones, cytoskeleton-related proteins, adhesion proteins, and various enzymes. Many of these proteins were also differentially expressed in comparative analyses of tumor versus non-tumor breast tissues. Gene set enrichment analysis revealed that the STIP1 interactome was predominantly enriched in pathways and biological processes related to the proteasome, protein folding, unfolded protein response, apoptosis, glycolysis, hypoxia, cytoskeleton organization, adhesion pathways, epithelial-mesenchymal transition, reactive oxygen species, and the G2/M checkpoint. The main enriched signaling pathways were associated with MTORC1, PI3K/Akt/MTOR, IL2/STAT5, and IL12. In summary, our study advances the understanding of the complex molecular patterns associated with breast cancer subtypes by exploring their differential proteomes and investigating the STIP1 interactome and its biological relevance. Further studies will be conducted to elucidate the precise molecular mechanisms underlying STIP1 expression and its interactions in breast cancer, which will provide deeper insights into tumor biology and identify potential biomarkers for improved diagnosis and treatment.

Agradecimentos: The authors would like to thank Federal University of Paraná, Hospital Nossa Senhora das Graças (HNSG) and Program for Technological Development in Tools for Health-

PDTIS-FIOCRUZ for providing the technical infrastructure and professional assistance. This work has been supported by the Brazilian research agencies CAPES and CNPq.