Elucidating The Role of Tumor Educated Platelets In Promoting Epithelial To Mesenchymal Transition And Angiogenesis In Breast Cancer: Therapeutic Intervention By Aspirin

Index No-109/22/Life Sc./28

Background: Despite existing reports highlighting role of platelets in tumorigenesis, its impact on breast cancer stem cells (BCSCs) remain underexplored. Our first ever report on murine and human system, accentuate that, tumor educated platelets (TEPs) of luminal-A and TNBC subtypes are distinct from healthy counterparts, collaborating with BCSCs to generate sub-variants that elevate tumor aggressiveness. While the pro-tumorigenic functions of platelets have been increasingly recognized, the role of platelet-poor plasma (PPP) remains underexplored in cancer biology. This study identifies PPP as a biologically active fraction of platelets with inhibitory effects on BCSC traits, including self-renewal and drug resistance.

Methods: Impact of TEPs on BCSCs was evaluated from primary breast tumor and blood samples of luminal-A/TNBC patients along with EC/4T1 murine breast tumor models and MCF-7/MDA-MB-231 cell lines. For downstream assays, TEPs were co-cultured with breast tumor samples or cell lines, followed by magnetic sorting of $CD44^{+}CD24^{-}$ BCSCs. TEP induced alterations of BCSCs were evaluated from 3D tumorsphere, colony formation, transwell migration, scratch-wound healing, matrigel invasion, *in-vitro* tube formation assays. Fluorescence-confocal microscopy, RT-PCR, flow-cytometry, western-blotting were utilized to decipher the role of genes and protein involved in stemness, metastasis along with the transcription factors in the downstream signaling cascade, followed by verifications by RNAi. Further, the influence of PPP on BCSCs was elucidated by co-culturing PPP with magnetically sorted $CD44^{+}CD24^{-}$ BCSCs of MCF-7 and MDA-MB-231. Using the 3D tumorsphere assay, colony formation assay and scratch-wound healing assay, PPP-induced changes to BCSCs were assessed. RT-PCR, flow-cytometry and ELISA were performed to investigate the changes in the expression of genes and proteins regulating stemness and drug resistance.

Results: TEPs have elevated expression of P-selectin and interacts with BCSCs via P-selectin and PSGL1 on BCSCs surface. Treatment with aspirin had restorative impact on P-selectin level, converting TEPs from active to resting platelet (RP) state. Under TEPs influence, BCSCs were tumorigenic, clonogenic, multidrug resistant, invasive with numerous invadopodia and remained skewed towards mesenchymal phenotype. Administration of RP or aspirin treated TEPs reduced TEP associated BCSC virulence both *in-vivo* and *in-vitro*. P-selectin-PSGL1 interaction resulted in binding of WNT to FRIZZLED followed by stabilization and nuclear translocation of β-Catenin. Nuclear β-Catenin promoted stemness-EMT-metastasis, along with stimulation of autocrine VEGF-VEGFR2 cascade. Inhibition of WNT and VEGFR2 by RNAi confirmed the critical role of this axis in regulating TEP's influence on BCSCs. In stark contrast, treatment with PPP resulted in a marked reduction in stemness markers *oct-4*, *sox-2* and marginally in *nanog*. Also, a prominent decrease in sphere-formation and migratory efficiency and sensitization towards chemotherapeutic agents, by downregulating the expression of ABC transporter genes like *abcb1* and *abcc1* was noted. This suggested an inhibitory effect on the cancer stem cell phenotype by PPP.

Conclusion: These insights into TEP-BCSC interplay, acknowledges TEPs, as-well-as unveils novel receptor-ligand signalling cascade, which could be a beneficial therapeutic strategy to target cancer metastasis. Aspirin treatment of TEPs markedly impairs their pro-tumorigenic functions in breast cancer. Aspirin irreversibly inhibits platelet cyclooxygenase (COX-1), preventing thromboxane A_2 production and platelet activation, which abrogates surface P-selectin upregulation and the secretion of metastasis-promoting factors. As a result, aspirin-treated TEPs lose their ability to induce EMT and stem-like traits in breast cancer cells. Mechanistically, aspirin disrupted the adhesive P-selectin/PSGL-1 interaction between TEPs and BCSCs effectively dismantling the protective TEP~CSC interfaces. Contrary to the supportive role of TEPs in tumor aggressiveness, PPP treatment led to a consistent and significant downregulation of stemness-associated genes, reduced mammosphere-forming ability and increased sensitivity to chemotherapeutic agents. These findings underscore the therapeutic relevance of PPP as a naturally occurring, platelet-depleted plasma fraction that inherently lacks tumor-promoting influence. The ability of PPP to suppress stem-like traits and overcome drug resistance in BCSCs points to a novel and underexplored avenue for therapeutic intervention. Strategically developing plasma-based therapies that neutralize or deplete platelet-derived tumor-supportive factors could represent a non-toxic, adjunctive strategy to sensitize tumors to conventional therapies and curb metastatic spread. In summary, this thesis not only reinforces the critical role of TEPs in breast cancer progression but also introduces platelet-poor plasma as a promising, tumor-suppressive biological medium. Further investigation into the molecular mechanisms underlying PPP's inhibitory effects may yield new biomarkers and therapeutic targets for effectively disrupting the cancer stem cell niche and improving long-term outcomes in breast cancer patients.

Saptak Banerjee 4/8/2025

Signature of the Supervisor

Dr. Saptak Banerjee, PhD
Senior Scientific Officer, Gr-I
Immunoregulation and Immunodiagnosis
Chittaranjan National Cancer Institute
Kolkata 700026

Aishwarya Guha 4/8/2025

Signature of the Candidate