Supplemental Methods

Volunteer Recruitment

Healthy volunteers over 18 years old, self-identifying as either AA or EA donated whole blood (~ 10 mL). All volunteers were interviewed on their medical history. Participants were asked for their self-identified race and age, and interviewed for their medical history to determine study eligibility. Health information, such as body mass index, blood pressure, serum lipids, and insulin resistance was not collected as part of the recruitment process. Exclusion criteria included history of cardiovascular disease (CVD), thrombosis, prior or current treatment for CVD or CVD risk factors, history of clinically significant bleeding, ongoing peptic ulcer disease or ongoing or acute gastritis, neurospinal disease, spinal surgery within 2 years prior to enrollment, active cancer diagnosis, and concomitant NSAID usage. Individuals who had taken NSAIDs or anti-platelet drugs within 5 days prior to blood collection were also excluded. Participants deemed eligible for study inclusion were provided an informed consent document.

Platelet isolation from whole blood

Whole blood was collected from healthy individuals following GWU IRB protocol (IRB approval number 021747). Methods adhered to IRB guidelines [1, 2]. Whole blood was spun at 170xg for 15 minutes to isolate platelet-rich plasma (PRP). Two hundred and fifty nM prostaglandin E1 (PGE1) and 1 mM EDTA were added to the PRP prior to centrifugation at 800xg for 15 minutes. The platelet pellet was washed with PBS containing 250 nM PGE1. Platelets were pelleted again by centrifugation at 500xg for 10 minutes. This wash step was repeated two additional times. Platelet purity was assessed by light microscopy and demonstrated no observable contamination by red blood cells or circulating nucleated cells.

Tumor Cell Induced Platelet Aggregation

MDA PCa 2b cells were obtained from American Type Tissue Collection (ATCC, Manassas, VA) and maintained in Kaighn's Modification of Ham's F-12 medium supplemented with 20% FBS,

25 ng/ml cholera toxin (Sigma-Aldrich, St. Louis, MO), 10 ng/ml mouse Epidermal Growth Factor (Corning), 100 pg/ml hydrocortisone (Sigma-Aldrich), 45 nM sodium selenite (Sigma-Aldrich), and 5 mg/ml human recombinant insulin (Life Technologies). PC-3 and LNCaP cells were obtained from ATCC and maintained in IMDM medium supplemented with 10% FBS. RC77 T/E cells were a generous gift from Johng Rhim and maintained in Keratinocyte-SFM medium (Gibco) supplemented with epidermal growth factor and bovine pituitary extract. All cell lines were maintained at 37 °C and 5% CO_2. For each cell line, 250,000 cells were resuspended in 50 μL of PBS. Platelet rich plasma (PRP) was collected from whole blood by centrifugation at 170× *g* for 15 min. Platelet density was determined using a hemocytometer. For each experimental group, 250,000,000 million platelets (in PRP) were centrifuged at 250x g for 15 min and resuspended 450 μL of PBS. Two hundred and fifty nM prostaglandin E1 and 1 mM EDTA were added as negative controls. Aggregation was tested using a Model 490 4+Four Channel Optical Aggregometer (Chronolog, Havertown, PA) according to manufacturer's protocol, as previously described by our group [1, 2]. Each channel of the instrument was set to 37 °C and magnetic stir bars were spun at 1200 rpm. A cuvette containing 500 μL of PBS was used as a blank control. The 450 μL platelet suspension was added to a respective cuvette with a magnetic stir bar. All channels were activated and start of the experiment was marked by a 50 μL addition of cell line suspension. The reaction was allowed to proceed for 10 minutes, after which data was extracted. Each optical density data point collected was graphed against time with 1 data point equaling half a second. Percent aggregation at a time point of 3 minutes was averaged over 3 biological replicates for each cell line to generate **Figure 2**.

1. Garofano K, Rashid K, Smith M, Brantner C, Suwunnakorn S, Diemert D, Gordon O, Horvath A, Khan S, Popratiloff A, et al. Prostate cancer cell-platelet bidirectional signaling promotes calcium mobilization, invasion and apoptotic resistance via distinct receptor-ligand pairs. Sci Rep 2023;13:2864. Epub 20230217.

2. Garofano K, Park CS, Alarcon C, Avitia J, Barbour A, Diemert D, Fraser CM, Friedman

PN, Horvath A, Rashid K, et al. Differences in the Platelet mRNA Landscape Portend Racial Disparities in Platelet Function and Suggest Novel Therapeutic Targets. Clin Pharmacol Ther 2021;110:702–713. Epub 2021/07/14.