Inhibition of platelet-tumor cell cross-talk by antiplatelet agents prevents the induction of a mesenchymal-like phenotype in HT29 human colon carcinoma cell

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Platelet-tumor cell interactions within the bloodstream play an important role in the metastatic dissemination of epithelial tumors [1]. The acquisition of a mesenchymal-like phenotype by colorectal cancer cells has been recognized as a relevant phenomenon in metastasis development [2]. The aims of this study were: (i) to explore the role of platelets on the expression of marker genes of epithelial mesenchymal transition (EMT) and the acquisition of migratory properties by human colon carcinoma cells (HT29); (ii) to verify whether selective inhibition of platelet cyclooxygenase (COX)-1 activity by aspirin prevents EMT and migration of HT29 cells; (iii) to assess whether these effects are shared by other antiplatelet agents, such as DG041 [antagonist of prostaglandin(PG)E_2 EP3 receptor] and ticagrelor ($P2Y_{12}$ receptor antagonist); (iv) to verify the role of thromboxane (TX)A_2 and PGE_2 in platelet-induced EMT. To these aims, washed human platelets ($1x10^8$) were added to HT29 cells ($1x10^6$) and cocultured for 20h or 40h (for gene or protein expression analyses, respectively) in presence of DG041 (3 μM) or ticagrelor (10 μM) or DMSO vehicle. As control condition, HT29 cells and platelets were cultured alone. The effect of aspirin was studied by the exposure of platelets to aspirin (300 μM) (to completely suppress platelet COX-1 activity); then, platelets were washed (to eliminate the drug) and added to HT29 cells. The conditioned media were collected for TXB_2 analysis (hydrolysis product of TXA_2) and PGE_2 by specific immunoassays; HT29 cells were washed to remove platelets and assessed for E-cadherin, Twist1 mRNA and protein levels (normalized to GAPDH) by qPCR and Western blot, respectively. The migratory properties of HT29 cells were assessed by Boyden chamber assay. Coculturing HT29 cells with platelets led to the downregulation of E-cadherin, a marker of epithelial phenotype, associated with the upregulation of Twist1 (transcription factor that down-regulates E-cadherin expression) [2,3]. Platelets increased the migratory capacity of HT29 cells *in vitro*. The EMT induction and migration of HT29 cells by platelets were inhibited by aspirin, DG041 and ticagrelor. During incubation of platelets with cancer cells, TXB_2 and PGE_2 were released (109±38 and 1.11±0.18 ng/mL, respectively) and aspirin profoundly inhibited their generation, suggesting their platelet origin. Since HT29 cells do not express detectable levels of TXA_2 receptor TP, but express EP receptors (EP1, EP2 and EP4, but not EP3), we hypothesized that platelet-derived PGE_2 was involved in the aspirin-sensitive mechanism responsible for platelet-induced EMT. In fact, exogenous PGE_2 (5nM, a concentration detectable in coculture medium) rescued the inhibitory effect of aspirinated platelets on the changes of E-cadherin expression. The inhibition of platelet activation by DG041 and ticagrelor, in platelet-HT29 cell cocultures, was associated with a significant reduction of TXB_2 and PGE_2 levels in the medium. The simultaneous inhibition of these prostanoids by DG041 or ticagrelor may suggest that antagonism of platelet EP3 or $P2Y_{12}$ signaling leads to reduced availability of arachidonic acid. In conclusion, antiplatelet drugs may exert an antimetastatic action by preventing EMT and migratory properties of cancer cells induced by the cross-talk with platelets. Our results provide a mechanistic understanding of the reported antimetastatic properties of low-dose aspirin in post-hoc analyses of randomized trials for cardiovascular prevention [4].

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