

Generation of 12- hydroxyeicosatetraenoic acid-phospholipid-esterified in platelet-cancer cell crosstalk

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Recently a new class of lipid mediators called oxidized phospholipids (oxLPs) has been identified in human platelets (Aldrovaldi et al., 2013). These mediators are generated through the enzymatic oxidation of membrane phospholipids, and they comprise eicosanoids attached to phosphatidylethanolamine (PE) and phosphatidylcholine (PC). Since platelets express two enzymes involved in the generation of eicosanoids, 12-lipoxygenase(LOX) and cyclooxygenase(COX)-1, it has been shown that they generate both hydroxyeicosatetraenoic acid (HETE) and prostanoid (PG)-containing phospholipids (O'Donnell et al., 2012). Upon thrombin activation, platelets produce 6 species of HETE-phospholipids: 2PCs (16:0a/, 18:0a/12-HETE-PC) and 4PEs (16:0p/, 18:1p/, 18:0p/, and 18:0a/12-HETE-PE). These esterified-HETEs remain cell-associated, and they can be externalized out of the plasma membrane (O'Donnell et al., 2012) thus regulating extracellular phospholipid-dependent signaling events. Moreover, these lipid mediators can affect the membrane dynamic during platelet activation and play a role in platelet vesiculation and/or degranulation (O'Donnell et al., 2012).

The interaction of platelets and tumor cells is recognized as a central event in the development of cancer metastasis. In the present study, we aimed to verify whether 12-HETE-phospholipids are produced during the crosstalk between platelets and HT29 colon adenocarcinoma cells. In particular, we aimed to assess 12-HETE-phospholipid generation in cancer cells, platelets and microparticles (MPs) isolated after 20 hours of coculture of washed human platelets (2×10^8) and HT29 cells (2×10^6). At the end of the incubation, platelets and MPs were collected from conditioned medium as previously described (Aldrovaldi et al., 2013) and HT29 cells were harvested by trypsin. MPs and cell pellets were extracted and analyzed for 12-HETE-phospholipids by LC/MS/MS analysis (Zhang et al., 2002). In platelet-cancer cell co-cultures, MPs were produced. To verify their cellular origin, platelets or HT29 cells were loaded with carboxyfluorescein succinimidyl ester (CFSE, $2 \mu\text{M}$, at 37°C for 10 min), and MP formation was assessed by flow cytometric analysis. Our data showed that MPs produced in platelet-HT29 cell cocultures were mainly derived from platelets. In fact, the number of platelet MPs and HT29 cell MPs averaged 6717 ± 1781 and 34 ± 1 (mean \pm SEM, $n=4$), respectively. In MPs, 12-HETE was esterified into phosphatidylethanolamine (PE) (18:0a; 18:0p; 18:1p; 16:0p) and phosphatidylcholine (PC) (16:0a; 18:0a). The most abundant 12-HETE-phospholipid was 12-HETE-PC (16:0a, 3.14 ± 0.63 ng/ml, mean \pm SEM, $n=7$). In the pellet of platelets cultured alone or cocultured with HT-29 cells, the most abundant product was 12-HETE-PC (16:0a, 46.75 ± 14.26 ng/ml and 35.61 ± 8.27 , mean \pm SEM respectively, $n=7$). In the pellet of HT29 cells cultured alone, 12-HETE-phospholipids were undetectable while in that of HT29 cells cultured with platelets, the major products were 12-HETE-PEs (18:0p and 16:0p, 75.93 ± 9.78 and 85.33 ± 11.6 ng/ml, mean \pm SEM $n=7$, respectively).

Altogether our results evidenced for the first time that during the interaction of platelets with cancer cells, 12-HETE is esterified in PE. Future work will determine whether these changes in plasma membrane composition of cancer cells, in response to platelet interaction, translates into enhanced migratory and invasiveness of tumor cells. This possibility will open the way to novel antimetastatic strategy involving the inhibition of 12-HETE-phospholipid generation in cancer cells.

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