Platelet release of PDGF causes cyclooxygenase-2 up-regulation in HT29 colon cancer cells: inhibition by imatinib mesylate

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Platelet-derived growth factor (PDGF) signaling is considered an important target for cancer treatment (1, 2). In cocultures of human platelets and HT29 colon cancer cells, we studied the role of PDGF released by platelets and downstream effectors of PDGF receptor (PDGFR) signaling on cyclooxygenase(COX)-2 expression in HT29 cells. Finally, we investigated the effect of imatinib, a PDGFR antagonist on COX-2 overexpression in this setting. Human HT29 cells (1x10⁶) were cultured alone or with isolated human platelets (1x10⁸) up to 20h. PDGF-BB, VEGF, EGF and active TGF-ß1 levels released in the medium were measured by immunoassays. The levels of COX-2 mRNA and protein were assessed in HT29 cultured alone or with platelets, by qPCR and Western blot, respectively. In HT29 cells cocultured with platelets, we assessed the effects of imatinib (10µM), a PDGF-neutralizing antibody (10µg/ml), wortmannin (PI3K inhibitor, 0.1µM), dm-amiloride [Na⁺/H⁺ exchanger(NHE) inhibitor, 10µM] and rottlerin (PKCd inhibitor, 10µM) on COX-2 protein induction. Nucleo-cytoplasmic translocation of the mRNA-stabilizing protein HuR was determined by confocal microscopy.

In co-culture of HT29 cells and platelets, we assessed the time-course of the release of different proteins from platelet α-granules. A substantial release of PDGF-BB and TGF-ß1 from platelets began after a lag-time of 4h. However, PDGF-BB levels were higher than those of TGF-ß1 and they continuously increased in a time-dependent fashion up to 20h. In contrast, TGF-ß1, after being released, decreased in a time-dependent manner suggesting its possible cellular re-uptake. At 2-4 h, platelets adhered to HT29 cells and the levels of COX-2 mRNA rapidly increased, and remained stable up to 20h. In contrast, COX-2 protein synthesis began to increase after a lag-time of 8h and, then, it continued to raise, in a time-dependent manner due to platelet-dependent induction of COX-2 mRNA stabilization associated with enhanced cytoplasmic accumulation of HuR. We provide several lines of evidence that platelet PDGF may be involved in posttranscriptional regulation of COX-2: i) the onset of PDGF-BB secretion occurred earlier than that of COX-2 protein synthesis; ii) the time-dependent increase of PDGF-BB levels was accompanied by a parallel upregulation of COX-2 protein; iii) imatinib and a specific anti-PDGF antibody prevented the induction of COX-2 protein; iv) pharmacological inhibition of downstream effectors of PDGFR, i.e. PI3K, NHE, and PKCd, by using wortmannin, dm-amiloride and rottlerin, respectively, reduced COX-2 protein induction.

In conclusion, these results showed that the interaction of platelets with cancer cells led to platelet release of PDGF which contributed to aberrant expression of COX-2. Imatinib affected this program of malignancy triggered by platelet-cancer cell cross-talk. Inhibition of PDGF-dependent induction of COX-2 by the drug may play a role in its anti-cancer efficacy.

References