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Non steroidal anti-inflammatory drugs and COX-2 inhibitors as anti-angiogenic drugs

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Non-steroidal anti-inflammatory drugs (NSAIDs) and specific inhibitors of cyclooxygenase (COX)-2, collectively referred to as COX-2 inhibitors, are therapeutics widely used for the treatment of pain, inflammation and fever. Experimental and clinical evidence indicate that COX-2 is a potential therapeutic target in cancer, and that NSAIDs and COX-2 inhibitors (COXIBs) also have anti-cancer activity.[1,2] COX-2 is expressed by cancer cells and cells of the tumor stroma during tumor progression and in response to chemotherapy or radiotherapy. In animals COX-2 overexpression promotes tumorigenesis while NSAIDs and COX-2 inhibitors suppress tumorigenesis and tumor progression. Clinical trials have shown that NSAIDs and COXIBs suppress colon polyp formation and malignant progression in patients with familial adenomatous polyposis (FAP) syndrome. Recent advances in the understanding of the cellular and molecular mechanisms of the anti-cancer effects of NSAIDs and COXIBs have demonstrated that these drugs target both tumor cells and the tumor vasculature.[3,4]

Tumor angiogenesis is an essential event for tumor progression and metastatic spreading.[5] NSAIDs and COXIBs inhibit angiogenesis through a combined inhibition of angiogenic growth factors production, response to angiogenic factor and impairment of endothelial cell survival and migration.[6]

We have originally shown that COXIBs block human umbilical vein endothelial cell (HUVEC) migration *in vitro* and angiogenesis *in vivo* by inhibiting integrin-mediated signaling events. Inhibition of COX-2 activity in endothelial cells by NSAIDs and COXIBS resulted in a diminished integrins $\alpha V\beta 3$-dependent activation Cdc42 and Rac, two members of the Rho family of GTPases that regulate cytoskeletal organization and cell migration, resulting in a decreased HUVEC spreading and migration *in vitro* and FGF-2-induced angiogenesis *in vivo*.[7] Direct pharmacological inhibition of integrin $\alpha V\beta 3$ was previously shown to inhibit angiogenesis in several experimental models.[8]

Subsequently we have reported that the COX-2 metabolite prostaglandin E2 (PGE2) accelerated $\alpha V\beta 3$-mediated HUVEC adhesion and promoted Rac activation and cell spreading, whereas thromboxane A2 (TXA2) retarded adhesion and inhibited spreading. We showed that the cAMP level and the cAMP-regulated protein kinase A (PKA) activity are critical mediators of the PGE2 effects. $\alpha V\beta 3$-mediated HUVEC adhesion induced a transient COX-2-dependent rise in cAMP levels, whereas the cell-permeable cAMP analogue 8-brcAMP accelerated adhesion, promoted Rac activation, and cell spreading in the presence of the COX-2 inhibitor NS-398. Pharmacological inhibition of PKA completely blocked $\alpha V\beta 3$-mediated HUVEC adhesion. A constitutively active Rac mutant (L61Rac) rescued $\alpha V\beta 3$-dependent spreading in the presence of NS398 or, but did not accelerate adhesion, whereas a dominant negative Rac mutant (N17Rac) suppressed spreading without affecting adhesion. PGE2 accelerates $\alpha V\beta 3$-mediated endothelial cell adhesion through cAMP-dependent PKA activation and induces $\alpha V\beta 3$-dependent spreading via cAMP- and PKA-dependent Rac activation.[9]

More recently we addressed the question of whether integrin-mediated cell adhesion may regulate COX-2 expression in endothelial cells. Cell detachment from the substrate caused rapid degradation of COX-2 protein in HUVEC independently of serum stimulation. This effect was prevented by broad inhibition of cellular proteinases and by neutralizing lysosomal activity but not by inhibiting the proteasome. HUVEC adhesion to laminin, collagen I, fibronectin, or vitronectin induced rapid COX-2 protein expression with peak levels reached within 2 h and increased COX-2-dependent prostaglandin E2 production. In contrast, non-specific adhesion to poly-L-lysine was ineffective in inducing COX-2 expression. Furthermore, the addition of matrix proteins in

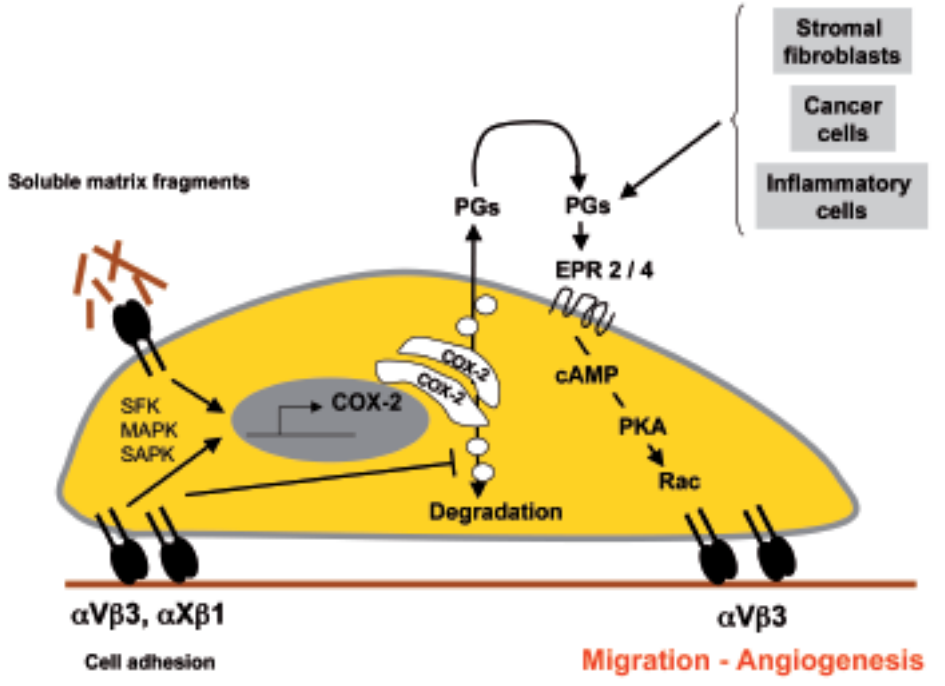

Figure 1. Proposed integrative model for the role of endothelial cell COX-2 in angiogenesis. Integrin mediated cell adhesion or binding of soluble ligands induce COX-2 mRNA expression and prevent COX-2 protein degradation. Endothelial cell, tumor and stroma-derived prostaglandin (PGE2) is required for αVβ3-dependent Rac activation, spreading and migration of endothelial cells. PGE2 binds and activate the G protein-coupled E prostane receptors 2 and 4 (EPR2/4), resulting in the rapid activation of adenylate cyclase (AC), transient cAMP raise and protein kinase A (PKA) activation, accelerated adhesion, Rac activation, spreading and migration. COX-2 activity also induces the expression of genes, but most of COX-2 targets in endothelial cells remain unknown to date. *SFK, Src Family Kinases; MAPK, Mitogen Activated Protein Kinases; SAPK, Stress Activated Protein Kinases.*

solution promoted COX-2 protein expression in suspended or poly-L-lysine-attached HUVEC. Adhesion-induced COX-2 expression was strongly suppressed by pharmacological inhibition of c-Src, PI 3-K, p38, ERK 1/2, and, to a lesser extent, PK C and by the inhibition of mRNA or protein synthesis. Thus, integrin-mediated cell adhesion and soluble integrin ligands contribute to maintaining COX-2 steady-state levels in endothelial cells by the combined prevention of lysosomal-dependent degradation and the stimulation of mRNA synthesis involving multiple signaling pathways.[10]

This work raises of course additional questions. A critical one concerns the identification of downstream target genes of the COX-2 pathway in endothelial cells or of genes that are suppressed by COXIBs. To this end we are performing a genome-wide screed for gene regulated by COX-2 and modulated by the Celecoxib in cultured HUVEC. Initial results indicate that transcripts encoding for cell adhesion receptors and signaling proteins, including some known to be active in transformed cells, are among the COX-2-regulated genes.

Furthermore, to validate the anti-angiogenic effects of COXIBs in patients, we have initiated a non-randomized, non-controlled, single center translational study aimed at demonstrating a correlation between the suppression of COX-2 activity, decrease in intratumoral PGE2 levels and the inhibition of angiogenesis, as assessed by measuring intratumoral and peripheral candidate markers of angiogenesis. The treatment begins the day after confirmed diagnosis of a resectable primary HNSCC in the oral cavity, hypopharynx or oropharynx and will end the day before definitive surgery (minimum of 14 days of treatment).

Unanticipated severe thrombotic cardiovascular complications were observed in a rofecoxib-based study in patients with arthritis and musculoskeletal pain (the VIGOR, Vioxx Gastrointestinal Outcomes Research, Study, 8076 patients). The meta-analysis of this study, revealed that patients without coronary artery disease had a 2.38 greater risk of developing a thrombotic cardiovascular event (myocardial infarction, unstable angina, cardiac thrombus, cardiac arrest, sudden death, ischemic stroke, and transient ischemic attacks) under rofecoxib treatment compared to patients treated with NSAIDs. In a similar study based on celecoxib (CLASS, Celecoxib Long-term Arthritis Safety, Study, 8059 patients), however, there was no

significant difference in cardiovascular event (myocardial infarction, stroke, and death) rates between celecoxib and NSAIDs-treated patients. The reasons for this increased risk in the VIGOR study are not fully clear and may be complex (including drug-specific effects). These data call for caution about the risk of cardiovascular events with COX-2 inhibitors. While, long-term preventive treatments with COXIBs are becoming difficult to justify, we should not forget the potential beneficial effects of COXIBs as therapeutic drugs in patients with declared cancers.

In conclusion, COX-2 has been recognized as an important molecule promoting tumor progression and angiogenesis. Preclinical and clinical studies indicate that COXIBs have anti tumor activities by directly targeting tumor cells and by suppressing tumor angiogenesis. The outstanding challenge is to demonstrate therapeutic efficacy of COXIBs in combination with chemo or radiotherapy, in well-designed clinical trials with clear endpoints.

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