Characterization of arachidonic acid -mediated signal transduction in regulation of NIH -3T3 cell adhesion to extracellular matrix

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Abstract

Cell-extracellular matrix (ECM) adhesion is characterized by discrete morphological and functional stages beginning with cell-substrate attachment, followed by cell spreading and then migration. These studies defined the lipid and protein signaling pathways regulating sequential transitions of adhesion stages NIH-3T3 cells on fibronectin (FN) substrate, emphasizing the signaling pathways stimulated by release of arachidonic acid (20:4, $\Delta^{5,8,11,14}$) (AA) from membrane phospholipids by phospholipase A2 (PLA2). Initial cell attachment to FN is via the extracellular domain of beta-1 integrin receptors. The beta-1 integrin cytosolic domain is required for receptor clustering and activation of PLA2; it also acts as the assembly site for focal adhesions formation to anchor the actin cytoskeleton. AA release is mediated by the 85 kD cytosolic PLA2 and not other PLA2's. Total AA release is rate-limiting in the overall process of adhesion, and is oxidized by lipoxygenases (LOX) or cyclooxygenases (COX) to generate adhesion signaling. During adhesion two functionally and kinetically distinct phases of AA release take place. An initial transient AA release is stimulated by cell attachment, and is sufficient to signal the cell spreading stage from 5-lipoxygenase (5-LOX) oxidation. LTB4, but not the cysteinyl LTs signals cell spreading. A later sustained AA release signals migration by its oxidation by upregulated cyclooxygenase-2 (COX-2). The second AA release and COX-2 protein synthesis are regulated by ERK-1/2. Constitutive overexpression of 5-LOX enhances l spreading and increases both rate and extent of actin polymerization, but limits motility. Increased LTB4 synthesis from 5-LOX stimulates f-actin polymerization and also increases total actin and beta-1 integrin expression. Constitutive overexpression of COX-2 slows cell spreading but increases motility. Upregulated COX-2 promotes disassembly of f-actin stress fibers, and induces redistribution of f-actin to permit motility, and decreases actin and beta-1 integrin expression. These data demonstrate a bifurcation in the AA adhesion-signaling pathway, wherein oxidation by 5-LOX signals actin polymerization regulating the spreading, while ERK 1/2-induced COX-2 synthesis generating prostaglandins signaling actin depolymerization and redistribution to enable migration. ^

Subject Area

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