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ACTIVATION OF PROTEIN KINASE C BY 12(S)-HETE: ROLE IN TUMOR CELL METASTASIS

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INTRODUCTION

Protein kinase C (PKC), the Ca^{2+} and phospholipid dependent kinase, belongs to the family of protein serine/threorine kinases. It is an important enzyme in mediating cell growth, cell differentiation and tumor promotion (1, 2). Physiologically, PKC is activated by diacylglycerol (DAG) generated during phosphatidylinositol (PI) turnover. Metabolism of PI is usually initiated by the action of phospholipase C in response to extracellular factors including hormones, growth factors and neurotransmitters. In addition to DAG, inositol trisphosphate (IP_3) is generated during PI turnover. While DAG activates PKC, IP_3 moblizes intracellular Ca^{2+}(3). Tumor promoting phorbol esters, such as phorbol 12-myristate-13-acetate (PMA), directly activates PKC by a mechanism similar to that of DAG (3).

12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE], a lipoxygenase metabolite of arachidonic acid, mimics the effect of PMA and increases surface expression of the αIIbβ3 integrin and enhances tumor cell adhesion to endothelium and subendothelial matrix (4). This implies a protein kinase C mediated mechanism of action for 12(S)-HETE. In fact, a number of fatty acids including arachidonic acid and some of its metabolites [including 12(S)-HETE] are able to directly activate protein kinase C *in vitro* (5-9). Recently we reported that 12(S)-HETE increased adhesion to endothelium of rat Walker 256 carcinosarcoma (W256) cells by activation of PKC (10).

Tumor cell metastasis is a multi-step process involving interactions among tumor cells and host cells (11, 12). We reported that subpopulations of cells, differing in their lung colonization potentials, could be isolated from B16a amelanotic melanoma (B16a) tumors by centrifugal elutriation (13). A positive correlation has been observed among lung colonization potential (13), ability to induce platelet aggregation (13), plasma membrane expression of αIIbβ3 integrin (14) and levels of cysteine proteinases cathepsin B and L (15, 16) in these B16a subpopulations. In this study, we compared the levels of PKC and 12(S)-HETE in these subpopulations. We also studied the effect of 12(S)-HETE on their adhesion to the matrix protein fibronectin. Finally, the involvement of PKC in adhesion to fibronectin was investigated by using protein kinase inhibitors.

MATERIALS AND METHODS

Materials.

12(S)-HETE was obtained from Cayman Chemical (Ann Arbor, MI). Staurosporine was from Biomol