Structure-function relationship

It has been shown that OxPLs can interact with G protein-coupled receptors such as the PAF-receptor or EP2 prostaglandin receptor (Marathe, 2001; Li, 2006). Furthermore, non-enzymatic oxidative cleavage of *sn*-2 residues can generate ligands for lysophospholipid receptors. However, structure-function relationship of OxPL-induced VEGF upregulation found in our experiments and discussed below does not support the involvement of any of these receptors.

First, we observed that the type of polar head group had minimal influence on induction of VEGF mRNA (Bochkov, 2006). This fact argues against the involvement of PAF receptor known to have strong preference for phosphatidylcholines (Shen, 1987). Furthermore, we used in our experiments synthetic *sn*-1-acyl-phospholipids, while the PAF-receptor is strongly selective for *sn*-1-alkyl-phosphatidylcholines (O'Flaherty, 1994). In addition, PAF-receptor preferentially binds phosphatidylcholines with short *sn*-2 residues (Shen, 1987), while in our experiments OxPLs containing long-chain isoprostane, hydroperoxide or hydroxyl residues demonstrated activity similar to or exceeding that of short-chain species such as POVPC or PGPC (Fig. 2, A). Second, we found that lysophosphatidylcholine (lysoPC) had dramatically lower activity in inducing VEGF as compared to phospholipids containing the *sn*-2 residue, thus clearly showing that these effects of OxPLs are unlikely to be induced by contaminating lysoPC acting via the G2A or other membrane receptors. Third, mass-spectrometry of oxidized phosphatidic acid (OxPAPA) showed that the preparation contained up to 5 molar % of lysophosphatidic acid (LPA) formed non-enzymatically during the oxidation (data not shown). Thus, micromolar concentrations of LPA were present during stimulation of cells with OxPAPA. However, these preparations stimulated expression of VEGF weaker than other classes of phospholipids that contained no LPA (Fig. 4 in Bochkov, 2006), thus making the involvement of LPA1-4 receptors unlikely. Fourth, our observation that the activity of OxPLs containing short-chain oxidized

groups such as POVPC, PGPC, phospholipid hydroperoxides or hydroxyls, was comparable to that of isoprostane-enriched OxPLs, argues against the involvement of prostaglandin EP2 receptor or other receptors specific for prostaglandins or isoprostanes. These negative results are further supported by the experiments with receptor agonists and antagonists, which are described in the main text.

Chromatin immunoprecipitation assay in HeLa cells

In order to ensure that the interaction of ATF4 with the "AsnSyn" site in VEGF promoter is not limited to the endothelial cells, we performed analogous experiment in cervical carcinoma HeLa cells. The data presented in Suppl.Fig. 6 demonstrate binding of ATF4 to the "AsnSyn" site in VEGF promoter suggesting that the ATF4 arm of UPR may be relevant for the induction of VEGF by OxPLs in non-endothelial cells as was described in our previous publication (Bochkov, 2006).

Supplementary references

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