Review

Low density lipoproteins and mitogenic signal transduction processes: Role in the pathogenesis of renal disease

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Summary. Abnormalities in lipid and lipoprotein metabolism are commonly observed in patients with chronic renal disease. Specifically, hyperlipidemia and the glomerular deposition of atherogenic lipoproteins (e.g., Low density lipoprotein, LDL; and its oxidized variants) are implicated in key pathobiological processes involved in the development of glomerular disease, including stimulation of monocyte infiltration into the mesangial space, mesangial cell hypercellularity, and mesangial extracellular matrix deposition. This review discusses recent understanding of glomerular mitogenic responses, intracellular signaling events associated with mesangial hypercellularity in renal diseases, and the participation of cholesterol and atherogenic lipoproteins in intracellular signaling pathways involved in mesangial cell proliferation.

Generally, the mitogenic intracellular signaling pathways are regulated by the activation of series of transmembrane and cytoplasmic protein tyrosine kinases that converge into the activation of Ras and down-stream mitogen-activated protein kinase (MAP kinase). Activated MAP kinase, through translocating into the nucleus and the activation of various transcription factors and protooncogenes, regulate cell proliferation. The importance of mitogenic intracellular signaling in mesangial proliferative disease has only recently been recognized and showed that the activation of MAP kinase and/or cyclin/cyclin-dependent kinases play crucial role in different phases of cell growth cycle and hypercellularity of glomerular cells in various experimental renal diseases. Using glomerular mesangial cells as an in-vitro model system, studies from our laboratory indicated that the accumulation of LDL and more potently its oxidized forms within the glomerulus, through the activation of membrane receptor tyrosine kinases (e.g., EGF receptor), activate Ras and MAP

kinase signaling cascade leading to DNA synthesis and subsequent mesangial cell proliferation.

These data suggest that atherogenic lipoproteins may act as one of the major endogenous modulators for mitogenic signaling response and cell proliferation within the glomerulus. It is reasonable to speculate that the correction or reduction of hyperlipidemia, glomerular lipid deposition, and the pro-oxidative milieu within the glomerulus, through the inhibition of mitogenic signaling events, may provide protective environment against mesangial hypercellularity and subsequent matrix deposition, and the progression of renal disease.

Key words: Atherogenic lipoproteins, Low density lipoproteins, Mesangial cell proliferation, Signal transduction pathways, Ras, MAP kinase, Glomerular disease, Glomerulosclerosis, Atherosclerosis

Background

Abnormal levels of lipids and lipoproteins are commonly associated with end-stage renal disease (ESRD), and are thought to contribute to the high incidence of cardiovascular events and mortality seen in ESRD patients. The common abnormalities in lipids and lipoproteins observed in renal disease vary considerably and include type IV and IIb hyperlipidemia that manifest hypertriglyceridemia, hypercholesterolemia, increased very low density lipoprotein (VLDL) and low density lipoproteins (LDL), and decreased high density lipoproteins (HDL) (Dieplinger et al., 1986; Grundy, 1990; Joven et al., 1990; Appel, 1991). ESRD Patients with hypertriglyceridemia have greater predominance of small dense LDL phenotype (Deighan et al., 2000), which are more atherogenic and exhibit increased risk of coronary artery disease as compared to buoyant LDL particles (Krauss, 1994; Gardner et al., 1996; Griffin et al., 1994). Analogous to human renal disease, hyperlipidemia is noted in various non-immune-

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mediated experimental renal diseases including renal ablation, models of diabetes mellitus, puromycin aminonucleoside- or adriamycin-induced nephrotic syndrome, Dahl salt-sensitive hypertensive rats and spontaneous or diet-induced hypercholesterolemic animals (reviewed in O'Donnell and Schmitz, 1991). Generally, in these experimental models, hyperlipidemia occurs in association with renal insufficiency, albuminuria, and accelerated development of glomerulosclerosis.

Although the association between lipid abnormalities and the pathogenesis of renal disease was recognized as early as 19th century, only recently this area of research has attained focus in understanding the nephro-toxicity of lipids and the identification and management of hyperlipidemia in patients with renal disease (Moorhead et al., 1982; Keane et al., 1988). The primary evidence linking hyperlipidemia and glomerular disease is predominately derived from experimental animal models of atherosclerosis. Cholesterol-feeding to experimental animals, an intervention used to induce accelerated atherosclerosis in herbivores caused the development of glomerular injury (French et al., 1967; Al-Shebeb et al., 1988; Groene et al., 1989; Keane et al., 1988; Diamond and Karnovsky, 1987). Furthermore, in obese Zucker rats, a genetic rat variant exhibiting endogenous hyperlipidemia, were shown to develop sequentially hyperlipidemia, proteinuria, mesangial expansion and subsequent glomerulosclerosis (Kasiske et al., 1985; Kamanna and Kirschenbaum, 1993). The treatment of hyperlipidemic animals with specific lipid-lowering drugs or modification in their dietary lipid intake could prevent or reverse the development of both glomerulosclerosis and atherosclerosis (Kasiske et al., 1988a,b). Despite the compelling evidence supporting a causal role for hyperlipidemia in experimental animals, similar relationships between hyperlipidemia and kidney disease have not been shown in humans. Comparative studies by meta-analysis showed that specific antilipidemic therapies generally had similar effects in different renal diseases when compared with hyperlipidemic patients without renal disease (Massy et al., 1995). Although these studies evaluated the efficacy of hypolipidemic agents and provided beneficial effects on lowering plasma lipid levels that may lower the risk for atherosclerotic coronary events in ESRD patients, the long-term benefits of treatment on the progression of either renal disease or atherosclerotic cardiovascular disease in ESRD patients are not clearly established. Previous prospective studies in humans have indicated an association between dyslipidemia and renal disease in patients with established renal disease (Sammuelsson et al., 1993, 1997, 1998; Washio et al., 1996; Hunsicker et al., 1997). Specifically, data from these studies showed that total cholesterol (Washio et al., 1996; Samuelsson et al., 1997), LDL-cholesterol (Samuelsson et al., 1997) and apo-B containing lipoproteins (Sammuelsson et al., 1993, 1997, 1998) are associated with the progression of renal disease. In the Modification of Diet in Renal Disease study, low HDL-cholesterol independently predicted the rate of renal decline (Hunsicker et al., 1997). Using participants of the Atherosclerosis Risk in Communities (ARIC) study, Muntner and associates have addressed whether abnormalities in plasma lipids are associated with the loss of renal function and the clinical onset of mild renal insufficiency (as assessed by a rise in serum creatinine of 0.4 mg/dl or greater) in subjects without established renal disease (Muntner et al., 2000). The data from this study indicated that high triglycerides and low HDL-cholesterol predicted an increased risk of renal dysfunction, and suggested that the treatment of these lipid abnormalities may decrease the incidence of early renal disease (Muntner et al., 2000). Although these data suggest causative role of lipids in early renal disease, the general consensus is that abnormalities in plasma lipids may modulate the progression, rather than the initiation, of renal disease. Since abnormalities in plasma lipids generally are secondary to renal disease, it is more logical to suggest that the abnormalities in plasma lipids in ESRD patients may further provoke the progression of renal disease. However, further investigation is timely in this area to understand the cellular and molecular mechanisms by which lipids and atherogenic lipoproteins modulate pathobiological processes involved in the progression of renal disease. More long-term clinical trials are warranted to address the role of hyperlipidemia in the progression of human renal disease and atherosclerotic coronary artery disease.

In this article, I will review recent advances in basic science regarding the role of lipids and atherogenic lipoproteins in collective pathobiological processes associated with the progressive glomerular disease, endogenous modulators of mesangial hypercellularity in glomerular diseases, mitogenic signal transduction processes associated with cell proliferation, and the participation of lipids and atherogenic lipoproteins in mitogenic signaling related to mesangial cell proliferation.

Role of lipids and lipoproteins in primary pathobiological processes associated with the progressive glomerular disease

Major cellular pathobiologic features involved in the development of diverse forms of glomerular disease include: 1) the enhanced accumulation of circulating monocytes within the mesangium, 2) the proliferation of glomerular mesangial cells, 3) and the exaggerated deposition of mesangial extracellular matrix proteins (ECM) within the glomerulus. The enhanced migration and accumulation of circulating monocytes within the mesangium in response to the injury or activation of the glomerulus are predominant cellular processes seen early in and before any histological glomerular abnormalities. The accumulation of monocytes and their transformation into tissue macrophages has been described in both immunologic- and nonimmunologic-mediated

glomerular injury in humans and various experimental models of renal disease (Schreiner et al., 1978; Magil and Cohen, 1989; Saito and Atkins, 1990). Beside monocyte infiltration, mesangial cell proliferation and accumulation of ECM proteins are characteristic features of progressive glomerulosclerosis. Glomerular mesangial cell proliferation has been suggested to be a critical histopathological event leading to the induction of consequent increased matrix deposition and glomerular sclerosis seen in various human renal diseases and experimental models of glomerular injury (Striker et al., 1991; Floege et al., 1993). Although, these pathobiological processes are universally seen in the pathogenesis of glomerular disease, the endogenous factors that initiate the cascade of cellular and molecular events involved in the development of glomerular disease are not clearly understood.

Because hyperlipidemia and the accumulation of atherogenic lipoproteins are commonly seen in patients with renal disease, studies from our and other laboratories have hypothesized that the accumulation of LDL and its oxidative modification within the mesangium may activate glomerular cells to produce various cytokines and peptides involved in monocyte infiltration, mesangial hypercellularity and exaggerated deposition of extracellular matrix proteins, the three major pathobiologic processes associated with progressive glomerular disease (Kamanna et al., 1997, 1998; Rovin and Tan, 1993). This hypothesis becomes even more relevant because the blood within the glomerular capillary is separated from the mesangium merely by a fenestrated endothelium without intervening basement membrane thus providing a conducive interactive tissue site for atherogenic lipoproteins and glomerular cells. Analogous to "Response-to-Injury" hypothesis for vascular disease, glomerular injury or mere activation processes (potentially mediated by cytokines, oxidative products, atherogenic lipids and lipoproteins, etc) may structurally alter the glomerular capillary endothelium allowing the leakage of circulating lipoproteins (such as LDL) and other macromolecules within the glomerulus, and which may in turn trigger various pathobiologic events in the glomerulus. Using in-vitro model system, we and others have performed series of studies to delineate glomerular cellular mechanisms by which LDL and its oxidized variants stimulate specific cytokines or peptides involved in glomerular monocyte infiltration and mesangial matrix deposition. The data indicated that the stimulation of glomerular endothelial cells with oxidized-LDL (ox-LDL) induced monocyte adhesion to glomerular endothelial cells mediated by increased expression of intercellular adhesion molecule-1 (ICAM-1, Kamanna et al., 1999a,b). Additional studies were designed to examine whether atherogenic lipoproteins modulate the synthetic properties of glomerular mesangial cells to produce monocyte chemotactic protein-1 (MCP-1) and monocyte colony-stimulating factor (M-CSF), specific cytoregulatory peptides implicated in monocyte

migration, differentiation and proliferation within the glomerulus. These studies indicated that the activation of glomerular mesangial cells with either native or ox-LDL markedly increased the expression of MCP-1 and M-CSF and induced monocyte migration and proliferation (Rovin and Tan, 1993; Pai et al., 1995; Kamanna et al., 1996). Although ox-LDL had a several fold greater activity, native LDL also induced significant mesangial cell M-CSF and MCP-1 and monocyte chemotaxis and proliferation. Further studies were performed to examine the effect of atherogenic lipoproteins on glomerular mesangial cell proliferation and extracellular matrix production. The activation of glomerular mesangial cells with LDL and ox-LDL induced mesangial cell proliferation (Ha et al., 1998) and mesangial cell extracellular matrix proteins (Ha et al., 1997; Roh et al., 1998). The effect of oxidatively-modified LDL was more pronounced in modulating cellular events than native LDL. These in-vitro studies clearly showed that the activation of glomerular endothelial and mesangial cells in response to atherogenic lipoproteins stimulated a series of cellular and molecular events associated with monocyte infiltration, mesangial cell proliferation and elaborated extracellular matrix expansion, characteristic histological features of many glomerular diseases. However, additional studies are needed to demonstrate the involvement of hyperlipidemia and glomerular atherogenic lipoprotein in key pathobiological processes involved in the progressive glomerular disease in in-vivo experimental models and human renal disease.

Role of lipids and lipoproteins in mesangial cell proliferation

An apparent increase in intraglomerular mesangial cell number has been noted in the early phases of many glomerular diseases characterized by progressive glomerulosclerosis (Striker et al., 1989 and references therein). Human and experimental glomerular diseases which appear to support these sequence of events, namely increased mesangial cell number followed by glomerulosclerosis include diabetic nephropathy, IgA nephropathy, membranoproliferative glomerulonephritis, and experimental models of anti-Thy 1-induced mesangial proliferative nephritis, puromycin aminonucleoside-induced nephrosis, and remnant kidney glomerular hypertension (Striker et al., 1989; Eng et al., 1994 and references therein). It has been suggested that the increased mesangial cell numbers in the early phases of glomerular disease may influence the synthesis and deposition of extracellular matrix (ECM) proteins leading eventually to sclerosis and renal failure (Eng et al., 1994 and references therein). Although the precise cellular and molecular mechanisms associated with mesangial hypercellularity in diverse glomerular diseases are not clearly defined, the stimuli for mesangial cell proliferation in response to glomerular injury have been attributed to multiple factors including growth factors, inflammatory mediators, immune

complexes, and complement components (reviewed in Striker et al., 1989; Abboud 1993; Eng et al., 1994). These factors involved in mesangial hypercellularity may be derived from intrinsic glomerular cells, acting in an autocrine or short-term paracrine manner, or from infiltrating inflammatory cells.

A growing body of evidence suggests that the abnormalities in lipid metabolism and accumulation of atherogenic lipoproteins in the glomerulus may serve as endogenous pathobiologic modulators for mitogenic signaling and mesangial cell proliferation. Hypercholesterolemia induced by high-cholesterol diet in experimental animals provoked mesangial hypercellularity (and monocyte accumulation and mesangial matrix deposition) and the development of glomerulosclerosis (French et al., 1967; Diamond and Karnovsky 1987; Al-Shebeb et al., 1988; Keane et al., 1988; Groene et al., 1989). Correction of hyperlipidemia in these experimental animals with lipid-lowering drugs markedly reduced mesangial cell hypercellularity and the development of glomerulosclerosis, suggesting a critical role for hyperlipidemia in mesangial cell proliferation (Kasiske et al., 1988a,b). Recent studies have indicated a pathological accumulation of lipids, LDL, and oxidized variants of LDL within the glomerulus of diverse human and experimental renal diseases (Avram, 1989; Lee et al., 1991; Sato et al.,

1991; Magil et al., 1993; Lee and Kim 1998). Increased mesangial deposition of apolipoprotein (apo) B and/or E (major proteins of LDL) seen in patients with primary and secondary glomerular disease was associated with mesangial hypercellularity, increased urinary protein excretion, and the development of glomerulosclerosis (Sato et al., 1991). Using in-vitro studies, data from several studies indicated that the stimulation of mesangial cells with LDL significantly increased mesangial cell DNA synthesis and cell proliferation (Wheeler et al., 1990, 1994; Grone et al., 1992; O'Donnell et al., 1993; Bassa et al., 1998). Although highly oxidized LDL was shown to inhibit human mesangial cell proliferation, we have shown that minimally-oxidized LDL, which is taken up by classic LDL receptors, significantly increased mesangial cell proliferation as compared to native LDL (Bassa et al., 1998). These in-vivo and in-vitro data suggest that the glomerular accumulation of LDL would be importantly involved in mesangial cell mitogenic responses. To highlight the relevance of cholesterol and LDL as endogenous modulators for mitogenic response, I have briefly reviewed below the simplified established mitogenic signal transduction paradigm and the potential interaction of cholesterol and LDL in this mitogenic signaling processes in order to delineate intracellular signaling mechanisms by which atherogenic lipoproteins
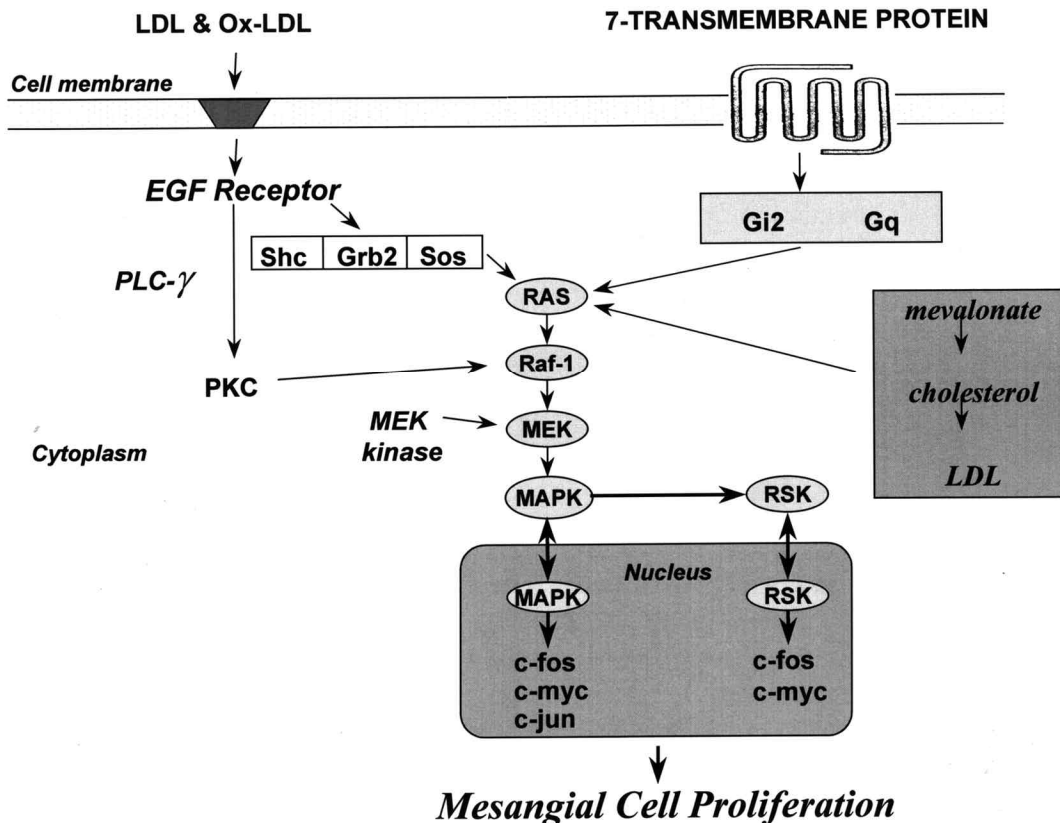

Fig. 1. Signal transduction paradigm for cell proliferation. Cellular mitogenic stimulus can induce the autophosphorylation and activation of intrinsic tyrosine kinase activity of specific membrane receptors, and which in turn can activate sequentially Ras, Raf, and MAP kinase. The activated cytoplasmic MAP kinase translocates into the nucleus and activate various transcription factors and protooncogenes associated with cell proliferation. PKC and G-protein-mediated events can also activate MAP kinase signaling. Atherogenic lipoproteins, by activating EGF receptor, may induce the activation of Ras-MAP kinase signaling. Increased stimulus for cholesterol synthetic pathway through farnesylation of Ras proteins may activate Ras and down-stream mitogenic signaling.

stimulate mesangial cell proliferation.

Established signal transduction pathways for cell proliferation

The intracellular signaling pathways involved in cell proliferation generally proceed in an orderly fashion by generating early multiple intracellular protein phosphorylation signals in the membrane and cytosol and within minutes, the mitogenic signal is propagated into the nucleus for DNA synthesis and cell multiplication. As noted in a simplified established model of mitogenic signal transduction paradigm (Fig. 1), cellular mitogenic stimulus (e.g., growth factors, serum, or possibly other endogenous mitogenic metabolic products) can lead to the autophosphorylation and activation of intrinsic tyrosine kinase activity of specific membrane receptors (e.g., EGF receptors, PDGF receptors, etc), and which in turn serves as high-affinity binding sites for the Src homology 2 (SH2) domain sequences that are encoded in different proteins (reviewed in Johnson and Vaillancourt, 1994). Shc and Grb2 adaptor proteins, containing SH2 domains, through phosphorylation by membrane receptors, interact with Sos, an exchange catalyst that stimulates GDP dissociation from Ras, allowing the GTP binding and activation of Ras. The activated Ras associates with the N-terminal regulatory domain of Raf protein kinases, leading to the activation of Raf kinase. Raf has been shown to be an integrator of signals received from various pathways including receptor tyrosine kinase and upstream serine-threonine kinases such as PKC, and through activating MAP kinase kinase (MEK) stimulates mitogen-activated protein kinase (MAP kinase) cascade. The activated cytoplasmic MAP kinase has been thought to translocate into the nucleus and activate various transcription factors and protooncogenes associated with cell growth and proliferation. Additionally, multiple signaling cascade including protein kinase C (PKC), protein tyrosine kinase (PTK), and G-proteins-coupled receptors may interact to activate Ras-MAP kinase cascade (Fig. 1). In late G1, the mitogenic signals discussed above converge with cyclin/cyclin-dependent protein kinases that regulate subsequent cell multiplication.

In addition to ligand-dependent phosphorylation and activation of membrane tyrosine kinase receptors (e.g., EGF and PDGF receptors), transactivation of EGF receptor by ligand-independent mechanisms has also been reported to participate in mitogenic signaling by various agonists (reviewed in Hackel et al., 1999; Moghal and Sternberg, 1999). For example, ligand-independent (in absence of EGF) phosphorylation of EGF receptor was noted in cells exposed to ultraviolet and gamma radiation, hydrogen peroxide, etc (Hackel et al., 1999). Besides the activation of receptor tyrosine kinases by these non-physiologic stimuli, EGF receptor was also shown to be phosphorylated/activated by G-protein-coupled receptor-mediated signaling (Hackel et

al., 1999). Studies have also shown stimulatory effects of various bioactive agents (such as, sphingosine and oxidative stress) on cellular transactivation of EGF- or PDGF-receptors (Davis et al., 1988; Nortwood and Davis, 1988; Gonzalez-Rubio et al., 1996). Additionally, intracellular calcium levels are shown to play a critical role in EGF receptor transactivation (Rosen and Greenberg, 1996; Zwick et al., 1997). In some studies, PKC has been shown to have a positive role in muscarinic acetylcholine receptor-induced EGF receptor phosphorylation (Tsai et al., 1997). Thus, the general consensus among established investigators in the area of mitogenic signaling is that the transactivation of membrane tyrosine kinase receptors (such as EGF receptor) plays a central role in integrating information from distinct multiple mitogenic agonist-mediated signaling (e.g., specific ligand-dependent and/or ligand-independent mitogenic modulators) leading to the activation of Ras-MAP kinase pathway and mitogenic responses. As discussed in later sections, we have obtained evidence to suggest such EGF receptor transactivation by atherogenic lipoproteins to modulate down-stream mitogenic signaling and mesangial cell proliferation.

Evidence of mitogenic signaling activation processes in experimental renal disease

Although mitogenic signaling pathways and mechanisms are not well defined in renal diseases, recent few studies have shown the modulation of mitogenic signaling in various experimental renal diseases. Increased activation of MAP kinase was noted in cortical and in isolated glomeruli of rats with proliferative glomerulonephritis induced by anti-glomerular basement membrane antibody or by anti-Thy1 antibody (Bokemeyer et al., 1997, 2000). Additionally, the expression and activation of specific cyclins and cyclin-dependent kinases are shown to be associated with various experimental renal diseases including mesangial proliferative glomerulonephritis, passive Heymann nephritis, remnant kidney, and tubulointerstitial disease (reviewed in Shankland, 1997). The regulatory role of mitogenic signaling including MAP kinase cascade and subsequent down-stream cell cycle processes in human renal disease has not been established.

Role of LDL and oxidized LDL variants in mitogenic intracellular signaling processes associated with mesangial cell proliferation

Using murine mesangial cells as an in vitro model system, we have previously reported data from series of studies examining the effect of LDL and minimally-oxidized/modified-LDL (mm-LDL, a proposed potent oxidized form of LDL within the vascular or glomerular tissue) on MAP kinase-Ras mitogenic signaling and mesangial cell proliferation. The data indicated that the stimulation of mesangial cells with either LDL or mm-

LDL for 24 h dose-dependently (2.5-20 μg/ml) induced mesangial cell proliferation (Bassa et al., 1998). The effect of mm-LDL was 2.1-2.8 fold greater when compared to controls. Specific tyrosine kinase inhibitors (e.g., genistein and herbimycin) significantly inhibited LDL/mm-LDL-mediated cell proliferation, suggesting the participation of tyrosine kinase signaling in LDLs-induced cell growth (Kamanna et al., 1999a,b). Additional studies indicated that both LDL and mm-LDL (10 μg/ml) increased cell membrane protein tyrosine kinase activity (as determined by an assay kit using a synthetic peptide of 12 amino acids surrounding tyrosine phosphorylation site in pp60src, specific for EGF receptor).

Effect of LDL and mm-LDL on EGF receptor phosphorylation and down-stream MAP kinase and Ras signaling

We further examined the effect of LDL/mm-LDL on the possible phosphorylation of cellular/membrane proteins. Preliminary studies indicated that LDL and mm-LDL (10 μg/ml) induced the phosphorylation of EGF receptors as early as 5-60 min (Kamanna et al., 1999a,b). Since LDL/mm-LDL-induced signaling occurred within 3-15 min of stimulation, it is very unlikely that the effect of LDLs could be due to growth factors secreted into the media. Additional studies were performed to examine the impact on down-stream Ras and MAP kinase signaling. Both LDL and mm-LDL (5-25 μg/ml) stimulated MAP kinase activity (10 min-30 min, and persisted up to 24 h), and the effect of mm-LDL was much higher than LDL (Bassa et al., 1998; Kamanna et al., 1999a,b). The incubation of mesangial cells with mm-LDL (10 μg/ml), as early as 3 min and up to 10 min, markedly stimulated the activation of Ras, and LDL had marginal stimulatory effects on Ras activation by 10 min incubation (Kamanna et al., 1999a,b).

Effect of lysophosphatidylcholine (LPC) on mesangial cell mitogenic signaling

LPC, one of the major components of oxidized variants of LDL has been implicated for various cellular responses of oxidized LDL. In order to identify potential active component of oxidized LDL for mitogenic signaling, additional studies were performed using LPC on Ras-MAP kinase signaling. We have shown that LPC (5-25 μM) activated MAP kinase and PKC in mesangial cells within 5-15 min of stimulation (Bassa et al., 1999). LPC-induced MAP kinase activation was significantly inhibited (but not completely) by prior cellular PKC inhibition, suggesting the participation of PKC and other additional signaling events (Bassa et al., 1999). LPC stimulated cell membrane protein tyrosine kinase activity, phospholipase C-1 phosphorylation, and markedly induced Ras activation within 2-10 min and sustained up to 60 min. These results indicate that LPC-

mediated PKC activation may be regulated by protein tyrosine kinase-dependent activation of PLC- 1, and both PKC and Ras pathways are involved in LPC-mediated down-stream MAP kinase activation. Because of the heterogeneous nature of oxidized LDL and its components, it may be likely that other potential components of oxidized LDL (in addition to LPC) may also be importantly involved in mitogenic signaling processes and mesangial cell proliferation.

Effect of cholesterol synthetic pathway intermediates on Ras-MAP kinase signaling and mesangial cell proliferation

Intermediary metabolites of cholesterol synthetic pathway are involved in cell proliferation. Lovastatin, an inhibitor of HMG-CoA reductase (a rate-limiting enzyme in cholesterol synthesis) blocks mevalonate synthesis, and has been shown to inhibit mesangial cell proliferation (O'Donnell et al., 1993). Recently, we have shown that the preincubation of mesangial cells with lovastatin inhibited the activation of Ras-MAP kinase signaling and mesangial cell proliferation stimulated by either serum or growth factors. Mevalonic acid and farnesyl pyrophosphate, but not cholesterol or LDL, significantly prevented lovastatin-induced inhibition of agonist-stimulated mitogenic signaling (Bassa et al., 1999). These results suggest that lovastatin, by inhibiting the synthesis of farnesol, a key isoprenoid metabolite of mevalonate, modulates Ras-mediated cell signaling events associated with mesangial cell proliferation. Additional studies also showed that the stimulation of mesangial cells with lovastatin decreased cell membrane Ras, and this effect of lovastatin was prevented by simultaneous exposure of mesangial cells to exogenous farnesyl pyrophosphate and geranylgeranyl pyrophosphate (Massy et al., 1999). Lovastatin reduced PDGF-induced mesangial cell DNA synthesis, and this was completely prevented by geranylgeranyl pyrophosphate (Massy et al., 1999). The in-vitro studies discussed above clearly indicate that the farnesyl and geranylgeranyl pyrophosphate, major isoprenoid metabolites of mevalonic acid in cholesterol synthetic pathway, are importantly linked to Ras and down-stream signaling events involved in mesangial cell proliferation. Furthermore, these observations suggest that lovastatin, in addition to lowering atherogenic lipoproteins (such as LDL), may provide additional benefits by inhibiting mitogenic signaling involved in hypercellularity of mesangial cells associated with diverse glomerular diseases.

Proposed model illustrating the participation of cholesterol and atherogenic lipoproteins in mitogenic signaling cascade associated with mesangial cell proliferation

Based on the in-vitro data from our studies, a workable model has been constructed to understand the

interaction of cholesterol and atherogenic lipoproteins in the established tyrosine kinase signaling pathway involved in mesangial cell proliferation (Fig. 1). As noted in figure 1, the accumulation of atherogenic lipoproteins (e.g., LDL and more potently its oxidized variants) within the glomerulus may stimulate the transactivation of EGF receptor and down-stream Ras-MAP kinase intracellular signaling events associated with mesangial cell proliferation. Alternatively, atherogenic lipoproteins, through the activation of phospholipase C-, may stimulate PKC activity and in turn activate Raf-1 and down-stream MAP kinase activity (Fig. 1). Increased stimulus for cholesterol synthetic pathway by farnesylation of Ras may activate MAP kinase pathway and cell mitogenesis.

Although the cellular mechanisms by which atherogenic lipoproteins or their active components activate specific membrane and/or cytosolic mitogenic signaling are not clearly established yet, several factors or processes may be proposed for the mitogenic signaling of atherogenic lipoproteins. For example, the cellular cholesterol deposition and/or the interaction of LDL and its oxidized forms, through membrane lipid-phase catalysis or causing perturbation in the lipid bilayer properties, may interact indirectly or directly with mitogenic membrane receptors resulting in the phosphorylation and kinase activation (Sargent and Schwyzer, 1986). The enhanced mitogenic signaling events of oxidized LDL or its components may be, in part, due to the impact of oxidative stress on cellular signaling. Additionally, the specific homology between cysteine-rich extracellular domains of growth factor receptors (e.g., EGF receptor and PDGF receptor) to the NH2-terminal extracellular domain of LDL receptor may have some contributory role in the interaction of LDLs with membrane receptors leading to the aggregation/autophosphorylation and down-stream events (Yamamoto et al., 1984 and references therein). As discussed earlier in the Signal transduction pathways for cell proliferation, the increased intracellular calcium levels and/or PKC activation by LDL and its oxidized products (Block et al., 1988; Scott-Burden et al., 1989; Yasenetskaya et al., 1993; Pahl et al., 1996) may stimulate the phosphorylation and transactivation of EGF receptor.

Thus, it is conceivable that the interaction of LDL with cells and/or increased stimulus for cholesterol synthetic pathway, through modulating some of the above noted mechanisms, may induce membrane receptor tyrosine kinase and Ras-MAP kinase mitogenic signaling cascade associated with cell DNA synthesis and subsequent cell proliferation (Fig. 1). In this paradigm, LDL and its oxidized products, intermediary metabolites of cholesterol synthetic pathway (such as farnesyl and geranylgeranyl), EGF receptor, PKC, and Ras-MAP kinase signaling appear to have a prominent role in signal transduction pathways involved in mesangial cell proliferation. Additional studies will be needed to confirm these interactions in in-vivo systems

so that they may act as the basis of new and novel treatment strategies to reduce or prevent lipid-mediated mesangial hypercellularity and subsequent glomerular disease.

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