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Review

Oxidation-Sensitive Transcription Factors and Molecular Mechanisms in the Arterial Wall

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ABSTRACT

Adaptation to various forms of cellular stress involves signal transduction into the cytoplasm and subsequently into the cellular nucleus, and ultimately alteration of gene regulation and expression. Increased oxidative stress, which is associated with increased production of reactive oxygen species and other radical species, plays a pivotal role in vascular dysfunction and contributes substantially to the structural and functional changes leading to vascular disease progression. Activation of oxidation-sensitive transcription factors and molecular mechanisms can be triggered in the systemic, tissue, cellular, and molecular environments, thereby affecting a multitude of pathophysiological events involved in the pathogenesis of atherosclerosis and other vascular diseases. Radicals *per se* also participate in the pathophysiological vascular response to shear stress and injury. Among the oxidation-sensitive transcription factors, important roles have been ascribed to nuclear factor-κB, c-Myc, and the peroxisome proliferator-activated receptor family. Regulation of nuclear events has also been recently proposed to involve corepressor and coactivator molecules. Identification of the genes that are involved in these processes has been facilitated by recent development of microarray chip techniques, which allow simultaneous evaluation of differential gene expression. As many of the transcription factors or their interactions are redox-regulated, antioxidant intervention may affect their bioactivity. Antioxid. Redox Signal. 3, 1119–1130.

INTRODUCTION

Oxidative stress has traditionally been viewed as a stochastic process of cellular damage resulting from aerobic metabolism, and antioxidants have been viewed simply as free radical scavengers. Only recently has it been recognized that reactive oxygen species (ROS; *e.g.*, superoxide anion, singlet oxygen, hydrogen peroxide, and hydroxyl radical) (26) are widely used as second messengers to propagate proinflammatory, growth-stimulatory, and several other unknown signals. This increasing knowledge has contributed to the corollary realization that oxidative stress and inflammation are interrelated and probably inseparable phenomena. New pharmacological strategies aimed at appropriately supplementing the antioxidant defense systems while antagonizing redox-sensitive signal transduction may allow improved clinical management of inflammatory or degenerative conditions, including vascular dysfunction, myocardial reperfusion injury, atherosclerosis, and Alzheimer's disease. Introduction of antioxidant therapies into mainstream medicine might be a

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possible and promising approach, but will require significant advances in our basic understanding of cell biology and clinical pharmacology. Comprehensive understanding of how ROS regulate mitogenesis and apoptosis in vascular smooth muscle and endothelial cells might permit development of novel strategies to modify or prevent vascular diseases in which these phenotypes may predominate.

Pathogenic processes and oxidation-related events influence several known and unknown transcription factors and their target genes in the arterial wall, where continuous interactions between different arterial cell types, and between the arterial wall and cellular and plasmatic components of the bloodstream, contribute to vascular dysfunction and atherogenesis. As many of these transcription factors or their interactions are redox-regulated, antioxidant intervention may affect their activity and may thereby modulate systemic, tissue, cellular, and nuclear oxidation-sensitive events. Although intracellular redox balance is tightly controlled in living organs, a shift in this balance leads to important cellular changes derived, at least in part, from a modification of the regulatory pattern(s) of gene expression. This phenomenon relies on many transcription factors whose activities are either increased or reduced by a pathophysiological rearrangement of the redox environment.

OXIDATIVE BALANCE IN THE CIRCULATING BLOOD

In health, there is a balance in the bloodstream between formation of oxidizing chemical species and their effective removal by endogenous plasma protective antioxidants. The cellular metabolism of oxygen in humans continuously produces small amounts of ROS and other free radicals. ROS include the hydroxyl radical, which is the most reactive and aggressive of the various ROS, superoxide radical, and singlet oxygen. Chemical reactions of these ROS can further lead to formation of potent oxidants like hydrogen peroxide and peroxynitrite. Under normal circumstances, the major source of ROS produced in the body derives from leakage of electrons from the mitochondrial and microsomal electron transport chains. ROS can induce oxidative damage to various biologic macromolecules including cellular membranes and lipoproteins by a process called lipid peroxidation, which is the autooxidation of polyunsaturated fatty acid side chains of lipids. Proteins may also be damaged by ROS, leading to structural changes or loss of enzyme activity. Furthermore, ROS can cause oxidative damage to DNA, leading to mutagenic lesions (19).

Endogenous antioxidant systems have developed to defend the body against ROS. Antioxidants might be defined as substances that, when present at low concentrations compared with those of the oxidizable substrate, significantly delay or inhibit oxidation of that substrate (26). The three main enzymes responsible for controlling ROS are superoxide dismutase, glutathione peroxidase (selenium-dependent enzyme), and catalase. Major nonenzymatic antioxidant defenses include vitamin E (of which α-tocopherol is the biologically and chemically most active form), vitamin C (the most prevalent antioxidant in circulating plasma), and β-carotene (the vitamin A precursor), as well as free metal and heme-binding proteins (ceruloplasmin, transferrin, haptoglobins, hemopexin, and albumin). Antioxidants can interfere in various levels of oxidative damage, from prevention to repair, and are therefore considered to operate at either primary, secondary, or tertiary levels. Primary defenses are considered to be those that prevent radical formation (*i.e.*, iron-binding proteins, such as transferrin and lactoferrin), whereas secondary defenses are those that remove or inactivate formed ROS (scavengers, vitamin E, vitamin C, and glutathione). Tertiary defenses operate to remove and repair oxidatively damaged molecules, and are particularly important for DNA integrity.

When the primary or secondary defense mechanisms are insufficient or inadequate, large increases in the quantity of ROS lead to increased oxidative stress, which is defined as a disturbance in the prooxidant–antioxidant balance in favor of the prooxidants, leading to a potential damage (68). Numerous pathological conditions are associated with increased production of radicals in plasma (11, 23, 24). Sources of oxidative stress include activation of the phagocytes (neutrophils, monocytes, mac-

rophages, or eosinophils), the immune system (the respiratory burst), release of iron and copper ions and metalloproteins, and the vascular damage caused by the process of ischemia–reperfusion. If the increase in oxidative stress is mild, the tissue often responds by producing accessory antioxidant defenses. However, severe oxidative stress can overwhelm the endogenous antioxidant defense systems and cause cell injury and death. Indeed, this mechanism has been proposed to underlie development of atherosclerosis in the vascular wall consequent to chronic exposure to cardiovascular risk factors.

Countering the effects of risk factors by maneuvers such as exercise, estrogen supplementation, and substitution of polyunsaturated fat for saturated fat is therefore effective in decreasing oxidative stress and beneficial in prevention of atherosclerosis (59). However, paradoxically, these maneuvers are also capable of increasing oxidative stress, because under certain pathophysiological conditions a prooxidant status shift could possibly be beneficial by promoting antioxidant/scavenger activities. Indeed, individuals with genetic deficiency in antioxidant/scavenger enzymes, those who respond poorly to prooxidant conditions, or those with overwhelming plasma/tissue oxidative stress may require exogenous antioxidant protection. This supplementation can profoundly affect the activity of oxidation-sensitive transcription factors. Recent data reinforce the concept that regular dietary intake of antioxidants blocks the progression of atherosclerosis, and that reduced oxidizability of plasma low-density lipoprotein (LDL) may represent a good marker to follow the action of these antioxidants (50, 55). The ability to monitor the efficacy of any antioxidant therapy with markers of oxidation may help direct interventional strategies and ensure the potential influence of antioxidants on coronary heart disease.

GENERATION OF OXYGEN RADICALS AND OTHER RADICAL SPECIES IN THE ARTERIAL WALL

In the arterial wall, radicals may be generated by cellular enzymes such as the NAD(P)H oxidase, xanthine oxidase, endothelial nitric oxide synthase, cyclooxygenase, myeloperoxidase, and lipoxygenase systems (25, 50, 51, 55). Cellular sources of radicals include bloodborne phagocytic cells, monocytes in the process of infiltrating the endothelial layer, as well as vascular smooth muscle cells, endothelial cells, and fibroblasts (25, 50, 55). Arterial cells are normally well protected from radicals by antioxidant defenses, *e.g.*, the oxygen-radical scavenger enzymes, but the rate of radical formation can exceed the local antioxidant defense capacity, resulting in increased oxidant stress (25, 50, 55). Indeed, similar to the circulating plasma, several pathological conditions are also associated with increased production of radicals in arterial cells (11, 23, 25). The consequences of enhanced oxidative stress in arterial cells are well documented *in vitro* and are generally consistent with the cell migration and proliferation, expression of proinflammatory cytokines and growth factors, and modification of the extracellular matrix observed in atherosclerotic lesions (64).

Increased generation of radicals may in turn be responsible for many aspects of vascular dysfunction and atherogenesis through modulation of the expression of genes that influence vascular reactivity, recruitment and deposition of LDL and inflammatory cells into the intima, smooth muscle cell proliferation, and vascular apoptosis. These pathophysiological events may then provide a positive feedback mechanism, for example, by stimulating production of radicals from enzymes. If the rate of radical formation overwhelms the local antioxidant defense capacity, a prooxidant shift in the arterial wall may ensue. This may further increase the oxidation of LDL, and consequent transformation of macrophages into lipid-laden foam cells may result from uptake of large amounts of oxidized LDL (oxLDL) via an entire family of scavenger receptors (71). The uptake of oxidized proteins or phospholipids via scavenger receptors can also lead to increased macrophage activation and generation of additional radicals. This may in turn promote the oxidation of minimally oxLDL into the extensively oxLDL, facilitate its uptake, and further increase oxidative stress. This vicious cycle is initiated and expedited by cardiovascular risk factors such as hypercholesterolemia or diabetic hyperglycemia, which contribute to

increase oxidative stress in the arterial wall (7, 32, 34, 42, 52, 56, 59, 61). Furthermore, many atherogenic effects of minimally and extensively oxLDL are exerted by modulation of gene expression and activation of selected transcription factors in arterial cells, often by the same pathways that are affected by intracellular redox radical formation. The oxidation of LDL may thus be both a consequence of and a cause for general or local prooxidant shift and increased oxidative stress within the arterial wall. However, to what extent extracellular oxidant stress may also directly influence the intracellular redox balance (for example, by depletion of cellular antioxidant defenses and scavenger enzymes) is still poorly understood.

OXIDATION-SENSITIVE CYTOPLASMIC AND NUCLEAR TRANSCRIPTION FACTORS

Activation of transcription factors and molecular mechanisms can markedly influence pathophysiological events associated with atherosclerosis and other vascular diseases. Several oxidation-sensitive transcription factors have been identified and attributed important roles in the cascade leading to oxidative damage to the arterial wall (46, 55). The most prevailing oxidation-sensitive transcription factor recognized is nuclear factor-κB (NFκB) (47). Because NFκB can be induced in many cells by a diverse set of stimulating substances, its activation has been proposed to be mediated via increased oxidative stress within the cell. However, this model was not found to be universal, because the association of NFκB activation and intracellular ROS generation has been detected only in certain cell lines. The origin of their interdependence is still unknown, but may well be situated in a particular kinase or in adaptator molecules of the signaling cascade, leading to inhibitor κBα (IκBα) phosphorylation. Cytoplasmic NFκB is activated by the cleavage of IκB from the p50–p65 heterodimer, which is then translocated to the nucleus. Cleavage of IκB requires an oxidizing milieu and appears to be one of the mechanisms through which radicals activate NFκB (28). On the other hand, NFκB can be activated by oxidants in several cell types, and this activation is well characterized at the molecular level especially in lymphocytes (28, 46, 47, 55). This activation is distinct from that of classical activators such as proinflammatory cytokines and phorbol esters, because the activation mechanisms appear to converge on a particular tyrosine residue of IκBα instead of the two classical N-terminal serines. The nature of the protein kinases or protein phosphatases involved in this process is still poorly understood. Other signaling pathways that activate NFκB include Ras (39) and the X chromosome-linked inhibitor of apoptosis protein (XIAP) (40).

Studies in cultured endothelial and smooth muscle cells demonstrated that multiple apoptotic signaling pathways are also influenced by radicals (13, 16, 37, 42, 54, 65, 66). Even mildly oxLDL, likely to be found in early atherosclerotic lesions, activates both Fas and tumor necrosis factor receptors (TNFR I and II pathways) (54), increases proapoptotic and decreases antiapoptotic proteins of the Bcl-2 family, and results in a marked activation of class I and II caspases (13, 54), which are among the end-effectors of apoptosis (Fig. 1). The role of radicals in this process is highlighted by the marked reduction of this activation in the presence of oxygen radical scavengers or antioxidants during LDL oxidation (13, 54). Several other important redox-regulated transcription factors include activated transcription factor-2 (ATF-2), Ets-like element kinase dependent 1 (ELK-1), cyclic AMP response element binding protein (CREB), activating protein-1 (AP-1), p53, and the c-Myc/Max complex and its binding factors, elongation factor 2 (E2F) and activating protein-2 (AP-2) (13, 14, 54) (Fig. 1). Some of these factors have been linked to apoptosis, such as p53 (10), whereas others appear to promote cell differentiation and proliferation, *e.g.*, AP-1 (46, 51, 55) and NFκB (47). Activation of "apoptotic" signaling appears to be accompanied by activation of growth-promoting transcription factors, such as NFκB, which may constitute a compensatory mechanism to limit cell death. Indeed, the extent of actual cell death observed in atherosclerotic lesions is much smaller than would be anticipated based on the prevalence of oxLDL in atherosclerotic lesions and the high percentage of TdT-medi-

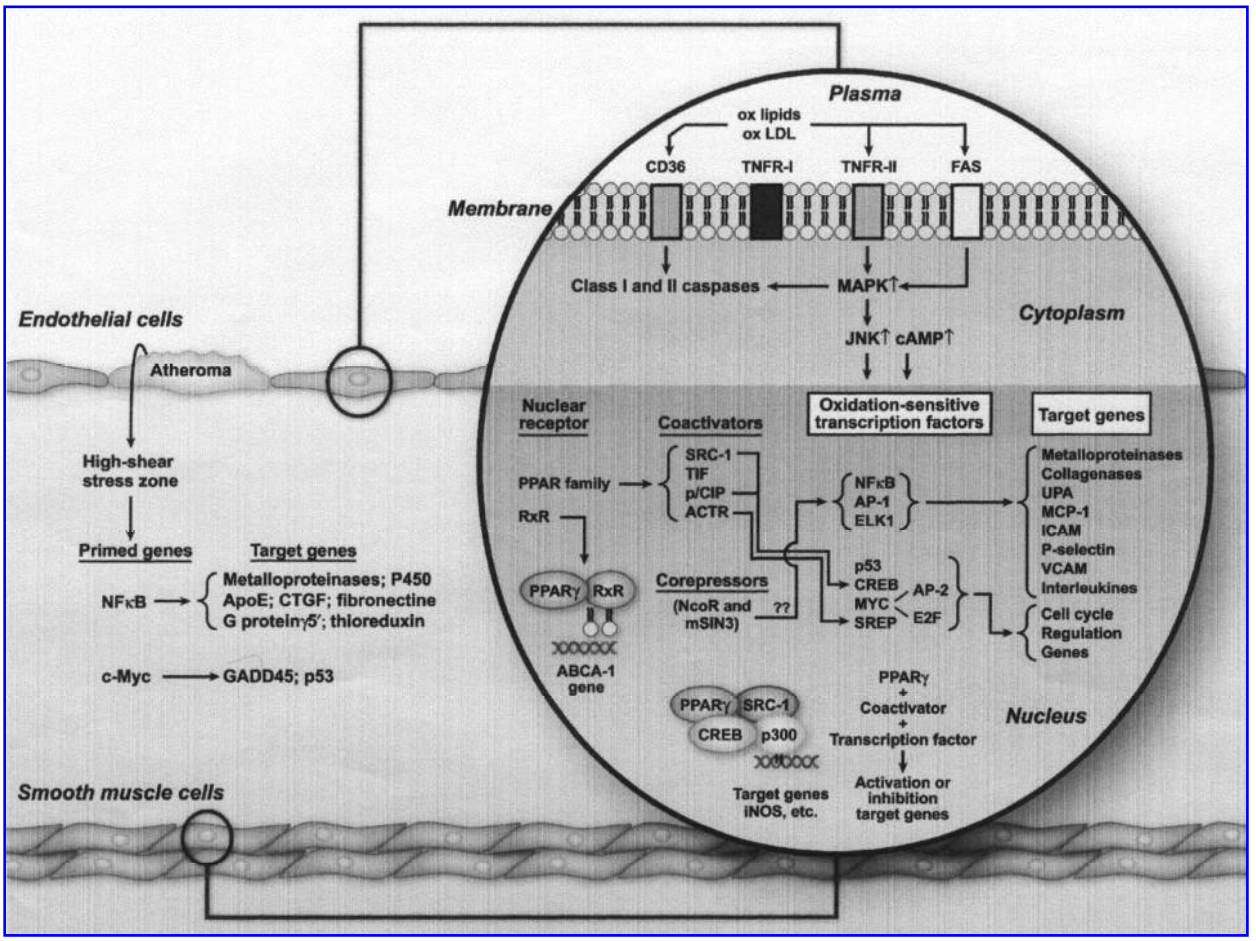

FIG. 1. A proposed pathophysiological scenario, in which cellular and nuclear oxidation-sensitive signaling and transcription pathways are depicted. On the cellular membrane are illustrated different receptors, through which the oxidized lipids can activate cytoplasmic events involving the kinase cascade and activation of several transcription factors. The target genes are divided into genes regulating cell cycle or those regulating the extracellular signaling. Oxidized LDL and phospholipids can activate nuclear receptors of the PPAR family by direct interaction or via membrane receptor CD36. PPAR heterodimerizes with RXR, modulating several target genes as ABCA-1. On the other hand, PPARγ can integrate with coactivator or corepressor molecules forming a bridge complex together with oxidation-sensitive transcription factors (CREB). High-shear stress induces priming of NFκB and c-Myc and their target genes in the arterial wall. These molecular interactions can subsequently affect the fate of vascular lesions. ApoE, apolipoprotein E; cAMP, cyclic AMP; CTGF, connective tissue growth factor; ICAM, intracellular adhesion molecule; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; MCP-1, monocyte chemotactic protein-1; SREP, sterol responsive element protein; UPA, urokinase plasminogen activator; VCAM, vascular cell adhesion molecule. (See text for other abbreviations.)

ated dUTP nick-end labeling (TUNEL) positive cells detected in lesions (38).

OxLDL can also foster foam cell formation by activating important nuclear receptors belonging to the peroxisome proliferator-activated receptor (PPAR) family. Peroxisomes in liver parenchymal cells proliferate in response to structurally diverse nonmutagenic compounds designated as peroxisome proliferators (PP) (51, 55, 76). Two mechanistic issues are important for consideration of this pathway: elucidation of the upstream events responsible for the tissue and species-specific induction of the characteristic pleiotropic responses by PPs, and delineation of the downstream events associated with peroxisome proliferation. The induction of peroxisome proliferation is mediated by PPARα, a member of a group of transcription factors that regulate the expression of genes associated with lipid metabolism and adipocyte differentiation (8, 9, 76). Three isotypes of this family of nuclear receptors, namely, PPARα,

PPARγ, and PPARδ (also called β), have been identified as products of separate genes (8, 9, 76). Although PPARα is responsible for the PP-induced pleiotropic responses, PPARγ seems to be involved in adipogenesis and differentiation. More specifically, PPARγ is a nuclear receptor that regulates fat cell development and glucose homeostasis (76) and is highly expressed in macrophage/foam cells of atherosclerotic lesions (63, 73). A striking colocalization of PPARγ and oxLDL has been reported in human and murine lesions (49, 63), and *in vitro* experiments confirmed that oxLDL up-regulates PPARγ expression (63). Interestingly, PPARγ activation *in vivo* could be either antiatherogenic, by down-regulating the expression of proinflammatory cytokines, or proatherogenic, by promoting macrophage expression of the scavenger receptor CD36 leading to foam cell formation. An *in vivo* study in LDL receptor-deficient mice treated with high doses of two distinct synthetic PPARγ activators indicated prevalence of the antiatherogenic effect (40). However, given other effects of these PPAR ligands (Fig. 1), in particular activation of the ATP-binding cassette transporter gene 1 (ABCA-1) (8), the impact of the down-regulation of inflammatory genes and up-regulation of CD36 on atherosclerosis needs to be investigated.

Enhanced oxidative stress is important not only in atherosclerosis, but also in vascular dysfunction or injury. Altered vasomotor regulation plays a central pathophysiological role in vascular diseases and could be partially reversed by antioxidant treatment. Shear stress *per se* can also trigger oxidation-sensitive gene expression (12, 33). Activation of c-Ha-ras by benzo(a)pyrene in vascular smooth muscle cell stress, and aryl hydrocarbon receptor (35) and increased oxidative reductase activity after balloon injury (72) have also been described. Finally, angiotensin II also stimulates a plethora of signaling pathways leading to cell growth and contraction. Recent studies have shown that ROS are involved in transducing many of the effects of angiotensin II, and are in fact produced in response to agonist-receptor binding (24). Angiotensin II stimulates a NAD(P)H oxidase to produce superoxide and hydrogen peroxide, both of which may act on intracellular growth-related proteins and enzymes to mediate the final physiological response. Hydrogen peroxide could mediate angiotensin II stimulation of important intracellular signal transduction such as epidermal growth factor-receptor transactivation, mitogen-activated protein kinase (MAPK), and activated kinase T (AKT). However, the precise redox-sensitive signaling pathways activated by angiotensin II, and their relationship to the physiology and pathophysiology of the renin–angiotensin system, remain to be fully delineated.

Interestingly, physical exercise also causes physiologically relevant redox changes in various cells and tissues (68). The physical exercise-induced molecular implications of such change are yet uncharacterized, including skeletal muscle contraction, cell adhesion, heat-shock proteins, programmed cell death, and carbohydrate metabolism.

GENE PRIMING

Location-specific priming of proatherogenic genes can play an important role in the propensity and ultimate development of cellular damage and atherosclerotic lesions in the arterial wall. NFκB in cultured endothelial cells was found to be activated by hemodynamic alterations induced by shear stress (2, 36). Similarly, increased NFκB activity was demonstrated in regions of altered flow, where hemodynamic forces influence variation in gene expression and may predispose these regions to atherogenesis if the appropriate systemic risk factors are present (48). Hemodynamic forces (*e.g.*, high shear stress) instigate a polygonal shape of the aortic cells in hypercholesterolemic mice, and these regions are subsequently highly predisposed to atherosclerosis and have strong activation of NFκB (27). Similarly, coronary arterial cells from lesion-prone regions in hypercholesterolemic pigs have enhanced c-Myc activity (15). Antioxidant intervention reduces this increased c-Myc activity and its target genes GADD45 (growth arrest DNA damage inducible gene 45) and p53. Taken together, these studies indicate that lesion-prone regions have "primed" genes, whose activation precedes development of atherosclerotic lesions.

COACTIVATORS AND COREPRESSORS

Recent studies demonstrated that transcription factors and nuclear receptors could be modulated by tissue-specific coactivator and corepressor molecules (Fig. 1). These molecules regulate the transcriptional function of nuclear receptors and mediate the interaction among some of the oxidation-sensitive transcription factors in a promoter-specific manner (22). For example, PPARs heterodimerize with 9-cis retinoic acid receptor (RXR), and bind to PP response element(s) on the target gene promoter to initiate inducible transcriptional activity (22). Tissue and species responses to PPs depend on pharmacokinetics, relative abundance of PPAR isotypes, nature of PP response element in the upstream regions of target genes, the extent of competition or cross-talk among nuclear transcription factors for PPAR heterodimerization partner RXR, and the modulating role of coactivators and corepressors on ligand-dependent transcription of PPARs (22). Using PPAR as bait in the yeast two-hybrid system, mouse steroid receptor coactivator-1 (SRC-1) and PPAR-binding protein (PBP) have been recently cloned; the best characterized are proteins of 160 kDa, including the coactivators SRC-1, glutamate receptor interaction protein-1 (GRIP-1), tissue initiator factor 2 (TIF2), peptide processing peptidyl glycine amidatin monooxygenase/COOH-terminal interactor protein-1 (p/CIP), activin receptor (ACTR), AIB1 (amplified in breast cancer (52, 54, 56–60) and several transrepressor molecules [glucocorticoid receptor (GR), RXR, thyroid receptor (TR)] (6, 67, 77) and the repressor complex containing nuclear receptor coactivator (NCoR), silencing nuclear protein repressor 3 (mSIN3), and histone deacetylase (29). Both SRC-1 and PBP contain LXXLL signature motifs, considered necessary and sufficient for the binding of coactivators to nuclear receptors. The identification of additional coactivators that may be responsible for cell-specific transcriptional activation of PPAR-mediated target genes, and generation of genetically modified animals (transgenic and gene-disrupted), will clarify the upstream and downstream targets responsible for the induction of early and delayed PP-induced pleiotropic responses. Mice deficient in fatty acyl-CoA oxidase, the first and rate-limiting enzyme of the peroxisomal β-oxidation system, revealed that this enzyme is indispensable for the physiological regulation of PPARα, and the absence of this enzyme leads to sustained transcriptional activation of genes regulated by this receptor. Moreover, the PPAR subfamily that may play a role in atherogenesis (19) can act together with LXR and, in concert with SREBPs, contribute to regulation of cholesterol homeostasis (62).

Several mechanisms have been described for transregulation of transcription factors. GR was demonstrated to negatively regulate NFκB, blocking its ability to bind DNA (67). Another mechanism for transrepression involves inhibition of signal transduction pathways necessary for activation of transcription factors. For example, activation of c-jun is inhibited by GR, RXR, and TR corepressors that inhibit the Jun terminal kinases, thereby preventing c-jun phosphorylation and formation of the AP-1 complex (6, 77). CBP and p300 are essential coactivators for a large family of oxidation-sensitive transcription factors and have been proposed as critical targets for the tranrepressive action of nuclear receptor in several pathophysiological conditions (1, 9, 29, 72, 74, 78).

MICROARRAY TECHNIQUES

Obviously, a large number of various target genes could be involved in the cellular response to specific types of stress. Determination of which of them are regulated, expressed, or involved in the cellular response to increased oxidative stress or antioxidant interventions could offer an exceptional opportunity to define the mechanisms involved in these processes. A novel methodological approach may now facilitate this quest and appears more favorable to achieve this goal than conventional differential display techniques. Indeed, recent progress in microarray techniques makes it possible to assess the mRNA expression of thousands of genes at a time (4, 21). Gene chips for 36,000 murine genes and expressed sequence tags are commercially available, and the technology to generate custom arrays comprising several hundred genes is also

beginning to spread. Gene-specific transcription activators are among the main factors, which specifically shape the transcriptome profiles. It is tempting to take advantage of their properties to decipher the genome expression circuitry. This offers a way to determine simultaneously a large spectrum of changes associated with increased oxidative stress, and to identify, by appropriate data mining strategies (17), clusters of genes regulated in analogous ways, irrespective of the pathway determining their expression. Moreover, the Human Genome Project has allowed considerable progress in the construction of physical and genetic maps and the identification of genes involved in human diseases. The accelerated accumulation of biological information and knowledge is due in large part to the sequencing projects of other organisms, which indeed paved the way for the Human Genome Project. The genetic profiles thus obtained should also permit the definition of new pathologic subclasses not recognizable by traditional clinical factors, as well as new markers for susceptibility to certain illnesses, and new prognostic markers or methods of predicting responses to treatment. With this experimental approach, it has been recently demonstrated that endothelial shear stress influences the differential gene expressions determining the functional phenotype (20). Such clusters can then be correlated with atherogenesis or vascular dysfunction, verified under different experimental conditions, and used to identify pathophysiological mechanisms leading to the progression or regression of the disease. Mice have become a favorite model for atherosclerosis research (58).

Obviously, applying microarray techniques to the arterial wall is complicated by the cellular heterogeneity and lack of synchronization of cell cycles. This may be overcome either by focusing on the normal arterial wall prior to the onset of intimal thickening, or by selectively extracting specific cells from defined stages of atherosclerotic lesions (Napoli *et al.*, unpublished observation), partly by laser capture microscopy (3). Figure 2 illustrates an optional use of mouse aorta for investigating differential gene expression in atherosclerosis and high shear stress regions. Because of the qualitative nature of the microarray analysis, there is need to corroborate the findings using other techniques (*e.g.*, northern blot, RT-PCR), and the challenge to manage and interpret the large

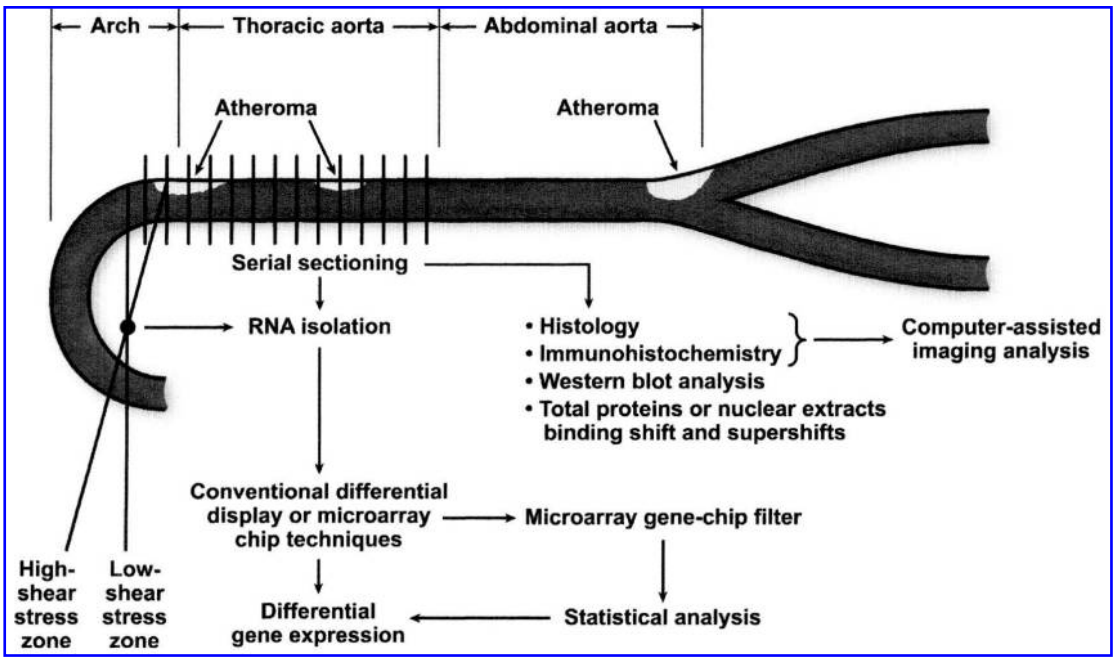

FIG. 2. Scheme of the use of mouse aorta for investigating differential gene expression in the pathogenesis of atherosclerosis, lesion-prone regions, and high shear stress regions by using differential display or micro-array techniques, and conventional molecular biology techniques, histological and immunohistochemical analysis.

data sets being generated implies the assistance of sophisticated statistical methods (5) and modeling (45).

CONCLUDING REMARKS

Increased oxidative stress is associated with increased production of ROS and several other radical species and activation of oxidation-sensitive mechanisms in the systemic, tissue, cellular, and/or molecular environments. These alterations may be involved in the pathogenesis of atherosclerosis and other vascular diseases and can contribute to disease progression. The resemblance of basic mechanisms implicated in the pathogenesis of various disease states (Fig. 1) may provide the potential for the development of a unifying framework concerning the etiology of the disease. New microarray chip techniques may allow for rapid screening and differentiation of candidate genes involved in various vascular diseases. Many transcription factors, receptors, or processes involved in these cascades are redox-regulated (including nuclear coactivator and corepressor molecules), and antioxidant intervention that modulates oxidation-sensitive events may therefore constitute a potentially useful and effective preventive or therapeutic strategy.

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ABBREVIATIONS

ABCA-1, ATP-binding cassette transporter gene 1; ACTR, activin receptor; AP-1 and AP-2, activating protein-1 and -2, respectively; CREB, cyclic AMP response element binding protein; E2F, elongation factor 2; ELK-1, Ets-like element kinase dependent 1; GADD45, growth arrest DNA damage inducible gene 45; GR, glucocorticoid receptor; IκB, inhibitor κB; LDL, low-density lipoprotein; MAPK, mitogen-actived protein kinase; mSIN3, silencing nuclear protein repressor 3; NCoR, nuclear receptor coactivator; NFκB, nuclear factor-κB; oxLDL, oxidized low-density lipoprotein; PBP, PPAR-binding protein; p/CIP, peptide processing peptidyl glycine amidation monooxygenase/COOH-terminal interactor protein-1; PP, peroxisome proliferator; PPAR, peroxisome proliferator-activated receptor; RXR, retinoic acid receptor; ROS, reactive oxygen species; SRC-1, steroid receptor coactivator-1; TIF2, tissue initiator factor 2; TNFR, tumor necrosis factor receptor; TR, thyroid receptor.

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