BACKGROUND

Transcriptional regulation by retinoids

Retinoids have profound effects on the regulation of cell growth and differentiation (3). Unlike the water-soluble hormones or growth factors, that bind to the cell-surface signaling receptors, fat-soluble hormones such as retinoids pass through the lipid bilayers of the cell membrane, after which they are free to interact with intracellular proteins. The biological effects produced by retinoids are believed to be mediated all or in part by two families of nuclear RA receptors (RARs) and retinoid X receptors (RXRs) (Fig. 2; Refs. 6, 7). Retinoids modulate certain gene expression directly through the binding to and activation of RARs and/or RXRs. The RAR and RXR gene family each comprises three subtypes, named α, ß, and γ (Fig. 2). They are ligand-dependent transcription factors that bind to *cis*-acting DNA sequences, called RA responsive elements (RAREs), in the promoter region of the target genes (Fig. 3A; Refs. 6, 7). It is well known that the expression of RARs increases immediately after stimulation with RA because RARs have a typical RARE sequence (8). RAREs are represented by two direct repeats (DRs) of the consensus AG(G/T)TCA core motif with different spacing (mainly DR5) between the two repeated half-sites. RARs bind to the RARE in response to both atRA and 9cRA, whereas RXRs bind and activate transcription in response to only 9cRA. To recognize a RARE, RARs usually must form a heterodimer with RXRs (Fig. 3A). RARs also suppress the activity of a transcription factor, AP-1, that is a complex of c-Jun and c-Fos, through trapping AP-1 and thus interfering its binding to the target DNA sequence called TAE (Fig. 3B; Refs. 9, 10). However, these two mechanisms explain the regulation of genes as much as 10% of whole retinoid-sensitive genes. Retinoids also regulate the expression of certain genes indirectly via several cytokines such as midkine (MK; Refs. 11-13),

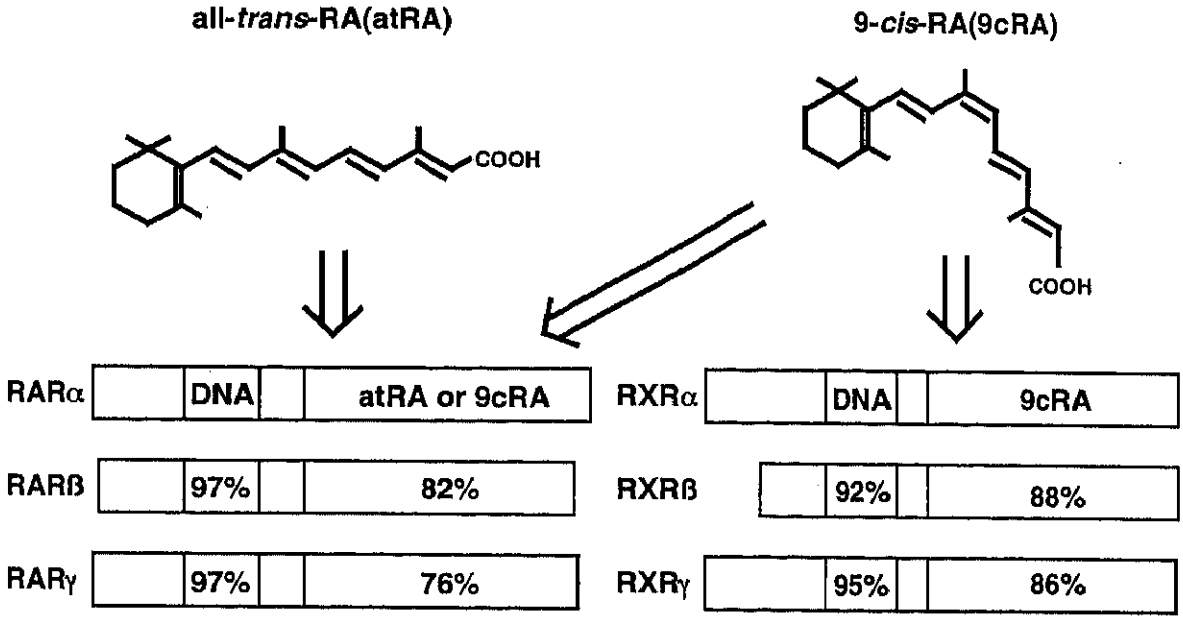

Fig. 2. Six types of retinoid receptors. The effects of retinoids are mainly mediated by two classes of nuclear retinoid receptors, RARs and/or RXRs. The RAR and RXR gene family each comprises three subtypes named α, β, and γ. atRA and 9cRA, two known active derivatives of vitamin A, essentially function as hormones by interacting with retinoid receptors. atRA binds to and activates RARs, and 9cRA is able to bind to and activate both RARs and RXRs.

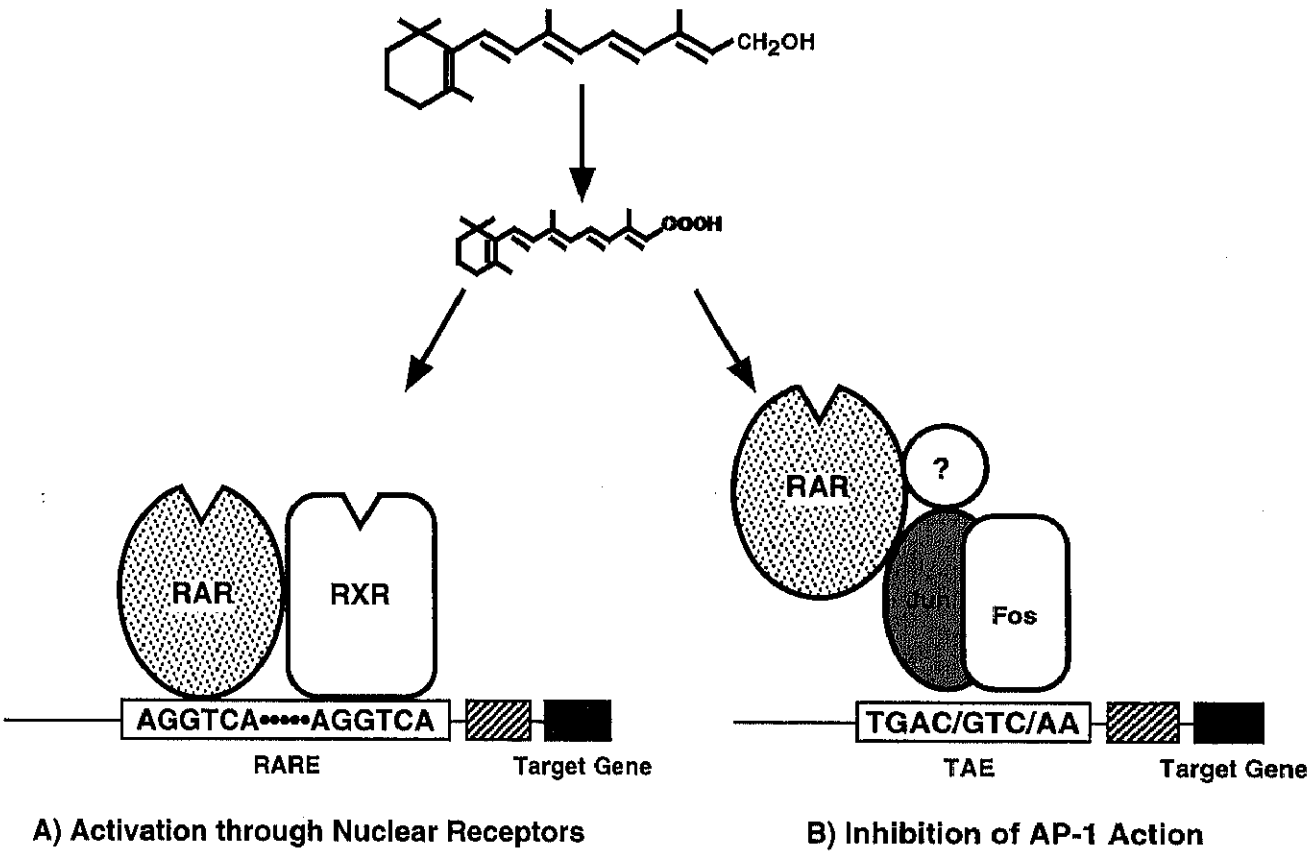

Fig. 3. Regulation of gene expression by retinoids through two different mechanisms. (A) RARs and RXRs are ligand-dependent transcription factors. They modulate the expression of target genes by interacting as either homodimers or heterodimers with RAREs. (B) RARs antagonize the activity of AP-1 that is composed of c-Jun and c-Fos, through protein-protein interaction. RARs interfere the binding of AP-1 to its cognate binding site, TAE, and snatch coactivators.

sonic hedgehog (14), and transforming growth factor-β (TGF-β; Refs. 3, 15).

Effect of retinoids on the endothelial cells

Vascular endothelial cells compose the inner layer of the blood vessels, and are considered as the first cell types to which circulating vitamin A interacts. As an important role of endothelial cells, they play key roles in the maintenance of fibrinolysis. They produce and secrete two immunologically distinct plasminogen activators (PAs), tissue-type PA (tPA), and urokinase-type PA (uPA), in different ratios depending upon the origin of the cells (16). Endothelial cells also produce and secrete an inhibitor of PA, PA inhibitor-1 (PAI-1), that rapidly inhibits the activity of both PAs (17). Hence, the balance between the production of PAs and PAI-1 from endothelial cells is important to maintain a normal fibrinolytic status. It is well known that endothelial cells play an important role in the angiogenesis, a process in which new blood vessels are formed, and that fibrinolytic activity is associated with this phenomenon (18, 19).

Several functions of endothelial cells are under the control of retinoids. An augmentation in collagen production was first reported (20). Successively, Kojima et al. have demonstrated that retinoids enhance the PA/plasmin levels of bovine aortic endothelial cells (BAECs) through up-regulating the expression of both uPA and its cell surface receptor (uPAR; Ref. 21). Retinoids also enhance the synthesis of tissue type II transglutaminase in BAECs (22). Furthermore, retinoids stimulate the expression of a heparin-binding growth/differentiation factor, MK, in BAECs (12, 13). MK participates in the augmentation of uPA expression and down-regulates the expression of PAI-1, resulting in a potentiation of retinoid enhancement of total PA/plasmin activity of the cells (12, 13). Because the activation of latent TGF-β is performed by surface plasmin,

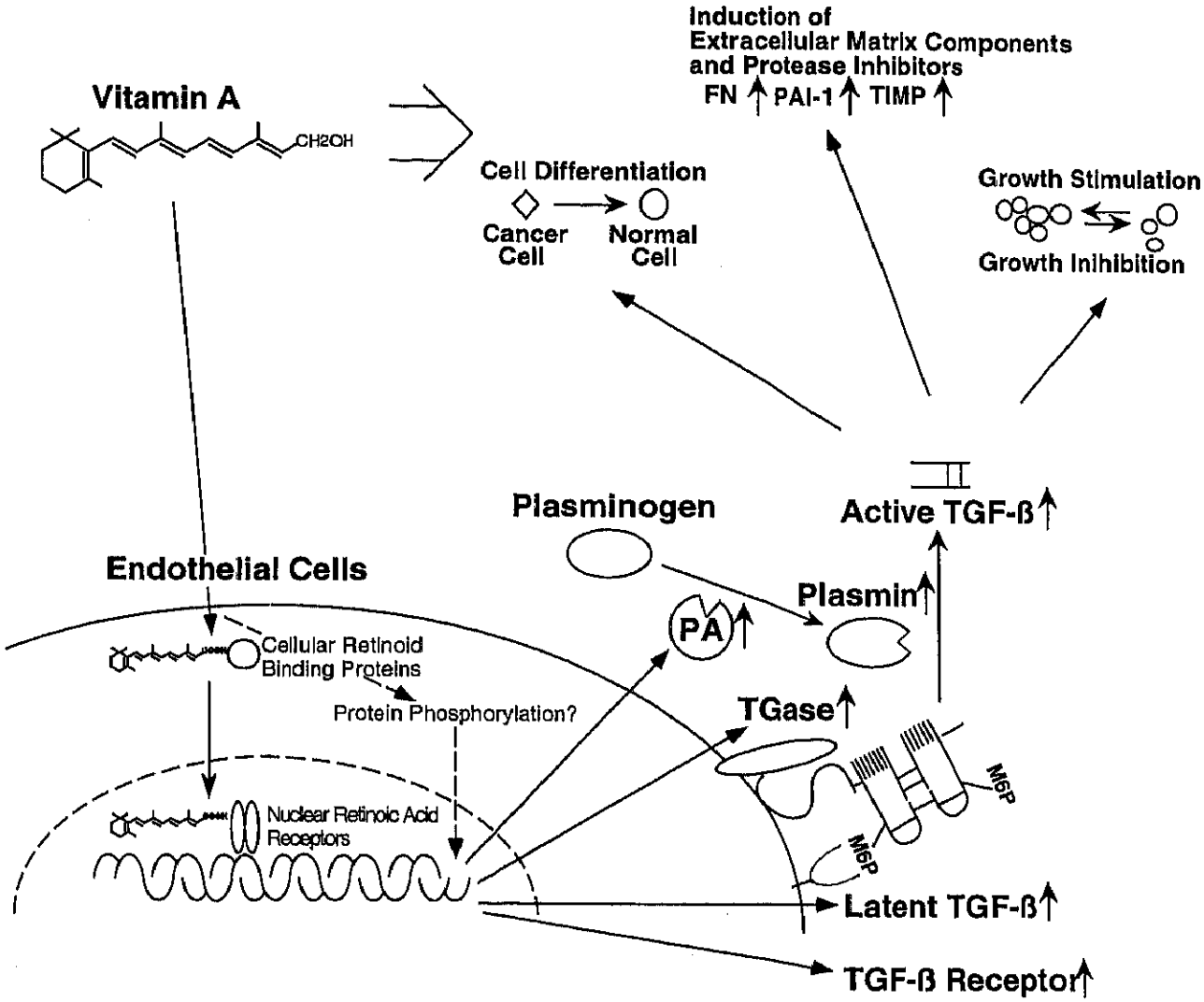

Fig. 4. Modulation of the endothelial cell functions by retinoids. Retinoids rapidly up-regulate the expression of both uPA and uPAR, and, subsequently, enhance cell-surface plasmin activity. Elevation of surface plasmin and transglutaminase levels by retinoids causes the formation of active TGF-β. The TGF-β generated acts as a mediator of retinoid actions such as suppression of cell migration and an enhancement of PAI-1 expression.

elevation of surface PA/plasmin and transglutaminase levels by retinoids causes the formation of active TGF-β (15, 23). Furthermore, retinoids up-regulate the expression of TGF-β signaling receptors (24). Therefore, at a focal site where retinoid is acting TGF-β generated mediates some of retinoid effects on endothelial cells such as suppression of cell migration (23) and an enhancement of PAI-1 expression (15). These are summarized in Fig. 4. Thus, retinoids may control the fibrinolytic level of endothelial cells both positively and negatively via MK and TGF-β, two biologically opposing cytokines (13). Moreover, several other changes induced by retinoids in endothelial cells are associated with the formation of TGF-β (25, 26), indicating a strong linkage between retinoids and TGF-β in endothelial cell biology. These sequential changes, induced in BAECs by retinoids, are initiated by up-regulation of the transcription of the uPA gene. However, its molecular mechanism has not been clarified yet.

Retinoids and the liver

The liver is composed of parenchymal cells (hepatocytes) and three different types of nonparenchymal cells, which include the sinusoidal endothelial cells, Kupffer cells (liver macrophages), and hepatic stellate cells (HSCs). The cellular elements of the liver are organized within the sinusoid, or microvascular unit, with the subendothelial space of Disse separating hepatocytes from the sinusoidal endothelium (Fig. 5). Hepatocytes, which account for approximately 66% of all cells and contain 90% of all proteins present in the liver, are directly involved in the synthesis and secretion of the mosts of blood circulating proteins. The much smaller and less abundant HSCs, which account for approximately 6-8% of all cells and contain 1%

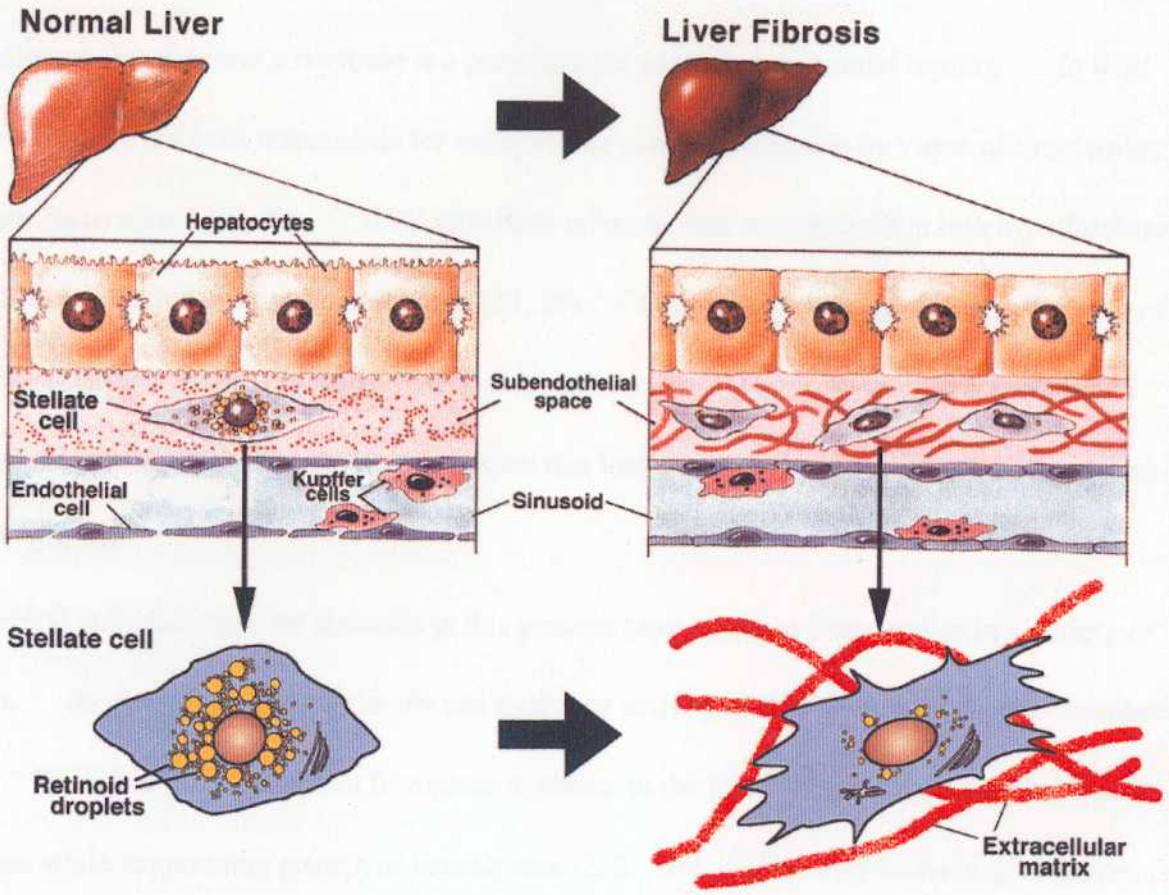

Fig. 5. Phenotypic changes of the HSCs during liver injury. During hepatic fibrosis, HSCs transform into myofibroblastic cells, characterized by increased proliferation and hyper-production of extracellular matrix, and lose their intracellular droplets of retinoid.

of all protein present in the liver, are the major storage cells of vitamin A. HSCs in normal liver are distinguished by prominent intracellular lipid droplets that contain vitamin A.

Liver fibrosis is a common response to chronic liver injury from many causes, including alcohol abuse, viral hepatitis (especially hepatitis B and C), and metabolic diseases due to metal overload. It is believed that the liver's response is a paradigm for parenchymal wound healing. In liver injury HSCs are the cells responsible for extracellular matrix production by virtue of their ability to undergo "activation" (27, 28). HSC activation refers to their transformation into myofibroblastic cells, during which they lose lipid droplets (27, 28). Major unresolved issues about the role of vitamin A (retinol) and its derivatives (retinoids) in liver fibrosis have been the mechanisms of intracellular retinoid loss and the potential roles this loss may play in facilitating HSC activation and hepatic fibrosis.

Several potential roles for retinoids in this process have emerged from studies in a variety of tissues. As described above retinoids can modulate activity of TGF-β in the vascular endothelial cells. TGF-β is the most potent fibrogenic cytokine in the liver, stimulating the production of collagen while suppressing growth of hepatocytes (2, 27, 29). It is very interesting to investigate whether during pathogenesis of liver fibrosis retinoids induce in HSCs similar sequential events as observed in the vascular endothelial cells.

ABBREVIATIONS

atRA: all-*trans*-retinoic acid

BAEC: bovine aortic endothelial cell

CAM: chorioallantoic membrane

EV: ethylenevinyl

GAPDH: glyceraldehyde-3-phosphate dehydrogenase

9cRA: 9-*cis*- retinoic acid

13cRA: 13-*cis*- retinoic acid

9,13dcRA: 9,13-di-*cis*- retinoic acid

GST: glutathione S-transferase

HSC: hepatic stellate cell

αMEM: α minimal essential medium

MK: midkine

PA: plasminogen activator

PAI-1: plasminogen activator inhibitor-1

PBS: phosphate-buffered saline

RA: retinoic acid

RAR: retinoic acid receptor

RARE: retinoic acid responsive element

RXR: retinoid X receptor

TGF-β: transforming growth factor-β

tPA: tissue-type plasminogen activator

uPA: urokinase-type plasminogen activator

uPAR: urokinase-type plasminogen activator receptor