

# The Roles of Proteinase-Activated Receptors in the Vascular Physiology and Pathophysiology

Katsuya Hirano

**Abstract**—Proteinase-activated receptors (PARs) belong to a family of G protein–coupled receptors, thus mediating the cellular effects of proteinases. In the vascular system, thrombin and other proteinases in the coagulation–fibrinolysis system are considered to be the physiologically relevant agonists, whereas PARs are among the most important mechanisms mediating the interaction between the coagulation–fibrinolysis system and the vascular wall. Under physiological conditions, PARs are mainly expressed in endothelial cells, and participate in the regulation of vascular tone, mostly by inducing endothelium-dependent relaxation. PARs in endothelial cells are also suggested to contribute to a proinflammatory phenotypic conversion and an increase in the permeability of vascular lesions. In smooth muscle cells, PARs mediate contraction, migration, proliferation, hypertrophy, and production of the extracellular matrix, thereby contributing to the development of vascular lesions and the pathophysiology of such vascular diseases as atherosclerosis. However, the expression of PARs in the smooth muscle of normal arteries is limited. The upregulation of PARs in the smooth muscle is thus considered to be a key step for PARs to participate in the pathogenesis of vascular lesions. Elucidating the molecular mechanism regulating the PARs expression is therefore important to develop new strategies for the prevention and treatment of vascular diseases. (*Arterioscler Thromb Vasc Biol.* 2007;27:27-36.)

**Key Words:** thrombin ■ receptors ■ vascular biology ■ endothelium ■ smooth muscle

Proteinase-activated receptor (PAR) is a G protein–coupled receptor, which mediates the cellular effects of proteinases. Since the discovery of the thrombin receptor, the first member of PARs in 1991 (later termed as PAR1),<sup>1</sup> three other members have been identified.<sup>2–4</sup> In endothelial cells, PAR1 contributes to endothelium-dependent relaxation or endothelium-dependent contraction, depending on the type of blood vessel (Figure).<sup>5–12</sup> PAR1 in endothelial cells also contributes to angiogenesis,<sup>6,9</sup> in addition to causing an alteration to the expression of multiple genes including cytokines, chemokines, and cell adhesion molecules (Figure).<sup>13,14</sup> In the smooth muscle cells, PAR1 mediates the contraction, cell migration, proliferation, hypertrophy, and production of the extracellular matrix (Figure).<sup>5–9</sup> Similarly, PAR2 also mediates endothelium-dependent relaxation and angiogenesis in endothelial cells, and mediates the contraction, cell migration, proliferation, hypertrophy and production of the extracellular matrix in the smooth muscle cells.<sup>5–9</sup> PAR3 functions as a cofactor for PAR4 activation, and therefore is not considered to directly elicit intracellular signals.<sup>9,15</sup> PAR4 has been reported to induce NO production in endothelial cells,<sup>16</sup> and an endothelium-dependent relaxation,<sup>17–19</sup> although its role in the smooth muscle cells remains unknown.

The proteolytic activation of the receptor is characteristic of PARs.<sup>5,8</sup> PARs are activated by cleavage at the specific site

of the N-terminal extracellular domain.<sup>5,8</sup> The resulting new N-terminal region then acts as a ligand to activate the receptor. Consequently, any proteinase that cleaves at such an activation site could thus serve as an agonist, however some proteinases are known to remove the ligand region, thus disarming PARs.<sup>8</sup> Table 1 summarizes both activating and inactivating proteinases for each member of PARs. It is important to note, some proteinases are listed as both activating and inactivating proteinases for the same receptor. Although controversial effects of proteinases have been reported with different types of cell and species, the precise mechanism for such contradictory effect remains largely unknown. The primary sequences of the extracellular region, the state of glycosylation of the receptor, and the difference in the kinetics of the enzymatic reaction at the different cleavage sites may influence the overall effect of proteinases on the receptor.

In the vascular system, thrombin and other proteinases in the coagulation–fibrinolysis system are considered to be the physiologically relevant agonists (Table 1). Other proteinases, such as mast cell tryptase, neutrophil cathepsin G, or T lymphocyte-derived granzyme A, are released from inflammatory cells and immune cells, and may also be relevant proteinases in the vascular system, especially in vascular lesions (Table 1). Thrombin activates PAR1 and PAR4 with

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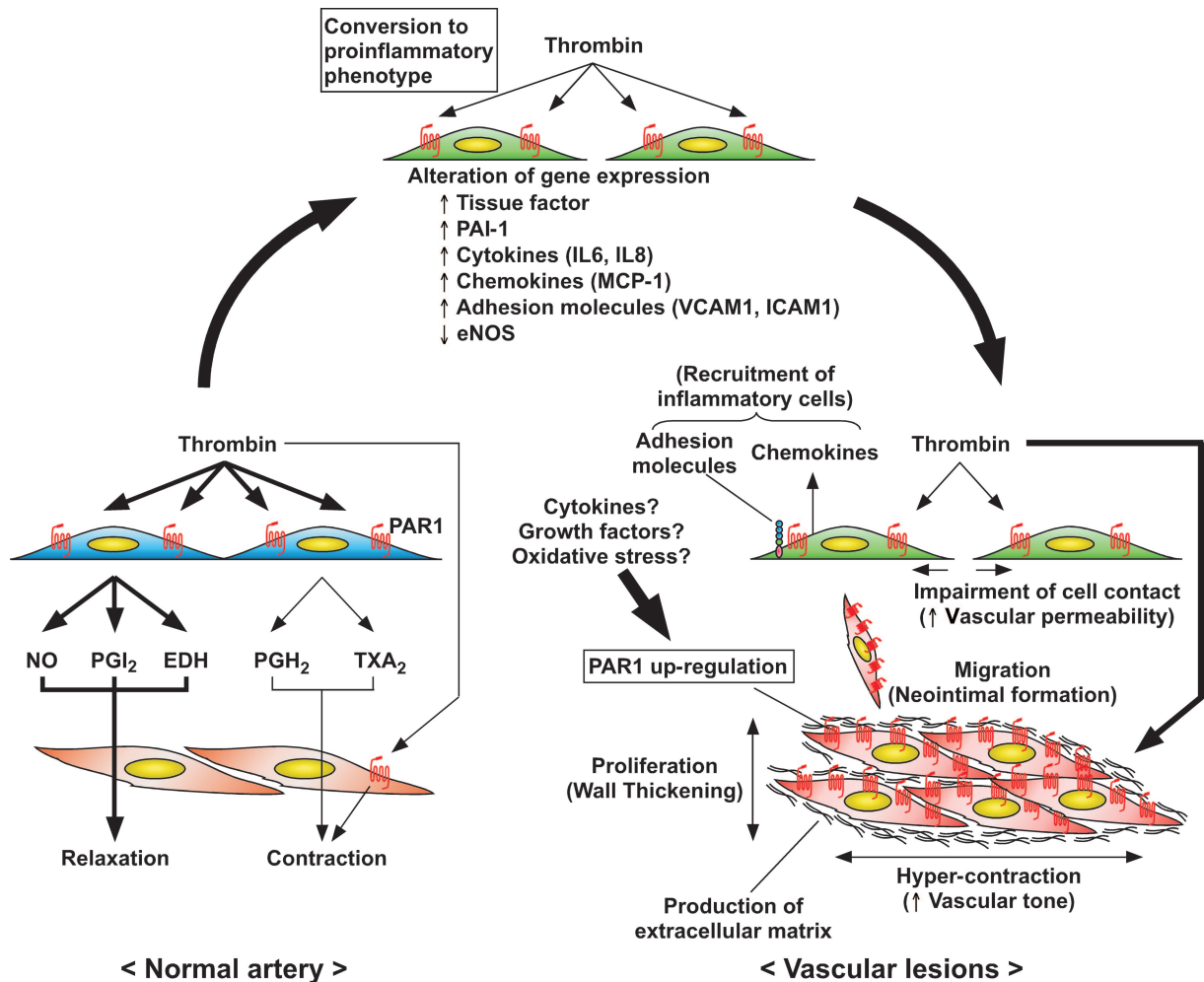
From the Division of Molecular Cardiology, Research Institute of Angiocardiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Correspondence to Katsuya Hirano, MD, PhD, Division of Molecular Cardiology, Research Institute of Angiocardiology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. E-mail khirano@molcar.med.kyushu-u.ac.jp

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The role of PAR1 in normal artery and vascular lesions. In a normal artery, thrombin induces endothelium-dependent vasorelaxation attributable to the production of nitric oxide (NO) or prostacyclin (PGI<sub>2</sub>), or endothelium-dependent hyperpolarization (EDH). Thrombin also induces endothelium-dependent vasoconstriction by inducing production of prostaglandin H<sub>2</sub> (PGH<sub>2</sub>) or thromboxane A<sub>2</sub> (TXA<sub>2</sub>), or directly contracts smooth muscle, depending on the type of artery. PAR1 in endothelial cells also causes an alteration of multiple gene expression, which is linked to the proinflammatory phenotype; PAI-1, plasminogen activator inhibitor-1; IL6, interleukin (IL)-6; IL8, IL-8; MCP-1, monocyte chemoattractant protein-1; VCAM1, vascular cell adhesion molecule-1; ICAM1, intercellular adhesion molecule-1. Such a phenotypic conversion was reported in the cultured endothelial cells. Its possible contribution to the early stages of vascular lesion development thus remains to be elucidated *in vivo*. In vascular lesions, the expression of PAR1 in smooth muscle is upregulated presumably by the action of cytokines, growth factors, or oxidative stress, etc. As a result, such smooth muscle effects of thrombin as contraction, migration, proliferation, hypertrophy, and production of the extracellular matrix become dominant in vascular lesions; contributing to an increase in the vascular tone, medial thickening, and neointimal formation in vascular lesions. On the other hand, an increase in vascular permeability mediated by the endothelial PAR1 also contributes to the pathophysiology of vascular lesions.

EC<sub>50</sub> of 50 pmol/L and 5 nmol/L, respectively.<sup>6,10</sup> The coagulation factors Xa and VIIa, in the complex with the tissue factor, activate PAR1 and PAR2.<sup>20-24</sup> Trypsin activates PAR1 and PAR2, but also disarms PAR1.<sup>2,25-28</sup> Plasmin either activates or disarms PAR1,<sup>6,29,30</sup> whereas it inactivates PAR2 and activates PAR4.<sup>31,32</sup> The activated protein C (APC) has been shown to activate PAR1 and PAR2,<sup>33-35</sup> although its functional role still remains controversial.<sup>36</sup>

In the following sections, I would like to discuss the role of PARs in regulating the vascular functions and in the pathophysiology of vascular diseases, with some focus on thrombin and its major receptor PAR1. For more information on the mechanism for receptor activation, intracellular signaling, and the role of PARs in other cell types, please refer to a number of comprehensive review articles.<sup>5,9,37-42</sup>

### Lessons From PARs Knockout Mice

The knockout mice for all members of PARs have already been established (Table 2). In *Par1*<sup>-/-</sup> mice, the birth rate (13.7%) obtained by mating *Par1*<sup>+/-</sup> was significantly lower than the Mendelian rate (25%).<sup>43,44</sup> Until gestational day 8, *Par1*<sup>-/-</sup> mice developed normally, and they were indistinguishable from their littermates. However, the growth of *Par1*<sup>-/-</sup> mice was retarded after gestational day 8, and about half of them died of hemorrhage during gestational days 9 to 10. The gross bleeding was associated with the abnormal development of the yolk sac vasculature, whereas there was no gross vascular malformation in the embryo proper.<sup>45</sup> However, the defects in the walls of great vessels were noted in the embryo proper, which were considered to be the source of bleeding.<sup>45</sup> Importantly, the restoration of the expression of

TABLE 1. The Activating and Inactivating Proteinases of PARs\*

	PAR1	PAR2	PAR3 †	PAR4
Activating proteinases				
Coagulation-fibrinolysis system				
	Thrombin <sup>1</sup>	Vlla <sup>21</sup>	Thrombin <sup>3</sup>	Thrombin <sup>4,85</sup>
	Plasmin <sup>29</sup>	Xa <sup>20,22,24</sup>		Plasmin <sup>31</sup>
	Vlla <sup>21</sup>	APC <sup>33</sup>		
	Xa <sup>22-24</sup>			
	APC <sup>33-35</sup>			
Others	Trypsin <sup>25,26</sup>	Trypsin <sup>2</sup>		Trypsin <sup>4,85</sup>
	Tryptase <sup>107</sup>	Tryptase <sup>20,107,112</sup>		Cathepsin G <sup>108</sup>
	Cathepsin G <sup>108</sup>	MT-SP1 <sup>113,114</sup>		Gingipain-R <sup>111</sup>
	Granzyme A <sup>26,109</sup>	Proteinase 3 <sup>114</sup>		
	MMP1 <sup>110</sup>	Acrosin <sup>20,115</sup>		
	Gingipain-R <sup>111</sup>			
Inactivating proteinases				
Coagulation-fibrinolysis system				
	Plasmin <sup>30,32</sup>	Plasmin <sup>32</sup>		
Others	Trypsin <sup>27,28,116</sup>	Cathepsin G <sup>32</sup>	Cathepsin G <sup>117</sup>	
	Cathepsin G <sup>32</sup>	Proteinase 3 <sup>32</sup>	Elastase <sup>117</sup>	
	Proteinase 3 <sup>32</sup>	Elastase <sup>32</sup>		
	Elastase <sup>32</sup>			
	Chymotrypsin <sup>25</sup>			

\*APC indicates activated protein C; MMP1, matrix metalloproteinase-1; MT-SP1, membrane-tethered serine protease 1.

†PAR3 acts as a cofactor for the activation of PAR4.<sup>15</sup>

PAR1 in the endothelial cells using the endothelium-specific promoter prevented the embryonic lethality.<sup>45</sup> These observations thus suggest that the defect of the PAR1 expression in the vascular endothelial cells (but not in other cell types) played a primary role in the bleeding and the resultant embryonic death. *Par1*<sup>-/-</sup> mice, which survived the critical period, developed normally and caught up with the heterozygotes to the point where they were indistinguishable.<sup>43,44</sup> In *Par2*<sup>-/-</sup>,<sup>46-48</sup> the birth rate lower than the Mendelian rate was noted in one report,<sup>46</sup> whereas the normal birth rate was observed in the other study.<sup>47</sup> Otherwise, *Par2*<sup>-/-</sup> developed normally to adulthood. *Par3*<sup>-/-4</sup> and *Par4*<sup>-/-49</sup> were not embryonically lethal and the mice developed normally to adulthood.

In adults, the hypotensive response to PAR1 and PAR2 stimulation was specifically lost in *Par1*<sup>-/-</sup> and *Par2*<sup>-/-</sup> mice, respectively, as expected.<sup>46</sup> However, as listed in Table 2, pathological processes were attenuated in some disease models of *Par1*<sup>-/-</sup> and *Par2*<sup>-/-</sup>. These observations thus suggested the crucial role of PAR1 and PAR2 in pathogenesis of such diseases (Table 2). Unexpectedly, the platelets derived from *Par1*<sup>-/-</sup> normally responded to thrombin.<sup>43</sup> *Par3*<sup>-/-</sup> platelets were found to be unresponsive to thrombin at lower concentrations (1 to 3 nmol/L).<sup>4</sup> However, they did respond to high concentrations of thrombin (10 to 30 nmol/L), although the response was delayed, but eventually reached a level comparable to that seen with wild-type at 30 nmol/L.<sup>4</sup> In contrast, *Par4*<sup>-/-</sup> platelets were totally unresponsive to thrombin, and *Par4*<sup>-/-</sup> mice exhibited bleeding diathesis.<sup>49</sup>

These observations suggested that mice platelets express PAR3 and PAR4, but not PAR1, as receptors for thrombin, and that PAR4 mediates the cellular effect of PAR3.<sup>4,15</sup> The thrombin-induced endothelium-dependent relaxation was markedly impaired in *Par1*<sup>-/-</sup> mice, whereas it was partly lost in *Par4*<sup>-/-</sup> mice.<sup>18</sup> These observations also suggest a dual receptor system in vascular endothelial cells in mice.<sup>18</sup>

### The Role of PARs in Vascular Physiology

In normal arteries, thrombin and trypsin have been reported to induce endothelium-dependent vasorelaxation,<sup>50-55</sup> endothelium-dependent contraction,<sup>56</sup> or direct smooth muscle contraction,<sup>54,55</sup> depending on the type of blood vessels and species (Figure).<sup>7</sup> In normal porcine and human coronary arteries, the activation of PAR1 induced an endothelium-dependent relaxation, while having no direct contractile effect on the smooth muscle.<sup>53,57</sup> In normal porcine interlobal renal artery, thrombin has been shown to induce a biphasic response in the vascular tone, consisting of the initial endothelium-dependent relaxation, followed by an endothelium-dependent contraction.<sup>56</sup> In normal guinea-pig aortas, rabbit aortas and canine coronary arteries, thrombin has been reported to directly contract smooth muscle.<sup>50,54,55,58</sup> However, it has also been shown to have no direct contractile effect in normal rabbit mesenteric arteries, rabbit femoral arteries, or rat aortas, while causing slight contractions in normal rabbit basilar arteries, at high concentrations (unpublished observations). As a result, the endothelial cells are considered to be the primary cells mediating the vascular

**TABLE 2. Phenotypes of PARs Knockout Mice**

<i>Par1</i> <sup>-/-</sup> <sup>43,44</sup>	Growth retardation with a delay in placental development at embryonic days 9–10 <sup>43</sup> Defect in the yolk sac vasculature, multiple bleeding and embryonic death in ≈50 % of <i>Par1</i> <sup>-/-</sup> , at gestational days 9–10 <sup>43–45</sup> Survivors develop to the level indistinguishable to <i>Par1</i> <sup>+/-</sup> and wild-type <sup>43,44</sup> Normal bleeding time, normal platelet response to thrombin <sup>43,44</sup> Partial defect in thrombin-induced endothelium-dependent relaxation <sup>18</sup> Attenuation of the vascular lesions development after arterial injury <sup>94</sup> Attenuated inflammation in experimental colitis <sup>118</sup> Tenuation of pulmonary edema in bleomycin lung injury <sup>119</sup> Reduced cerebral infarction size in experimental transient focal cerebral ischemia <sup>120</sup>
<i>Par2</i> <sup>-/-</sup> <sup>46–48</sup>	A birth rate lower than the Mendelian ratio in one report <sup>46</sup> normal birth rate in the other <sup>47</sup> No gross abnormalities <sup>46,47</sup> Attenuated inflammatory responses in surgical trauma, <sup>48</sup> experimental arthritis, <sup>47</sup> experimental infectious colitis, <sup>121</sup> experimental autoimmune encephalomyelitis <sup>122</sup> Reduced allergic inflammatory response in airway <sup>123</sup>
<i>Par3</i> <sup>-/-</sup> <sup>4</sup>	No embryonic lethality No gross abnormalities
<i>Par4</i> <sup>-/-</sup> <sup>49</sup>	Normal bleeding time, partial defect in platelet response to thrombin No embryonic lethality No gross abnormalities Impaired hemostasis, complete defect in platelet response to thrombin Partial defect in thrombin-induced endothelium-dependent relaxation <sup>18</sup>

effects of PARs in normal arteries (Figure).<sup>59</sup> On the other hand, the functional expression of PAR1 and PAR2 in the smooth muscle cells of normal arteries appear to be limited.<sup>60,61</sup> Their effect on smooth muscle may thus play a minor functional role in normal arteries.

### PARs-Mediated Production of Nitric Oxide in Endothelial Cells

Nitric oxide (NO) plays a major role in PARs-mediated endothelium-dependent vasorelaxation.<sup>6,7,18,53,62,63</sup> The NO production by endothelial NO synthase (eNOS) is primarily dependent on the Ca<sup>2+</sup> signal.<sup>64</sup> In line with this, thrombin-induced NO production is reported to be sensitive to calmodulin inhibitors.<sup>65</sup> The relationship between the extent of elevation of cytosolic Ca<sup>2+</sup> concentrations ([Ca<sup>2+</sup>]<sub>i</sub>) and the amount of NO production, has been reported to vary with the type of stimulant present. Thrombin has been shown to induce greater NO production at a given elevation of [Ca<sup>2+</sup>]<sub>i</sub> than that seen with ATP, bradykinin, or ionomycin.<sup>65</sup> These observations suggest that thrombin activates Ca<sup>2+</sup>-independent as well as Ca<sup>2+</sup>-dependent mechanisms of NO production in endothelial cells.

The activity of eNOS has previously been shown to be regulated, in a Ca<sup>2+</sup>-independent manner, by the phosphorylation of eNOS, and an interaction with regulatory proteins such as heat shock protein 90 and caveolin.<sup>64,66</sup> The phosphorylation of eNOS at Ser1177 (in human) has been shown to activate NO production in a Ca<sup>2+</sup>-independent manner.<sup>67,68</sup>

In addition, phosphorylation at Ser615, Ser633,<sup>69</sup> and Tyr817<sup>70</sup> has been reported to be associated with an increase in NO production, whereas phosphorylation at Ser495 was associated with a decrease in NO production.<sup>71</sup> The changes in the state of eNOS phosphorylation could thus contribute to the Ca<sup>2+</sup>-independent component of thrombin-induced NO production. However, thrombin has been reported to inhibit the phosphorylation of eNOS at Ser1177 in a manner dependent on the RhoA–Rho kinase pathway.<sup>72</sup> Furthermore, long-term treatment with thrombin was shown to downregulate the expression of eNOS in endothelial cells.<sup>72,73</sup> Alternatively, a dual receptor system for thrombin in the endothelial cells (PAR1 and PAR4)<sup>18</sup> may explain the thrombin-induced Ca<sup>2+</sup>-independent and dependent production of NO. The stimulation of PAR4 has been reported to induce NO production without a concomitant elevation of [Ca<sup>2+</sup>]<sub>i</sub> in endothelial cells. The PAR4-mediated NO production was also found to be resistant to the intracellular Ca<sup>2+</sup> chelator BAPTA.<sup>16</sup> The PAR4-mediated Ca<sup>2+</sup>-independent NO production may support the Ca<sup>2+</sup>-independent component of the thrombin-induced NO production.

### The Role of Endothelial PARs in Vascular Pathophysiology

Proteinases in the coagulation–fibrinolysis system and other proteinases including those derived from inflammatory and immune cells, are activated under pathological conditions such as thrombus formation, hemorrhage, inflammation, or

**TABLE 3. The Situations Associated With the Alteration in the Expression of PARs in Vascular Endothelial Cells and Smooth Muscle Cells**

Situations Associated With Altered Expression of PAR	Changes in the Expression	Type of Cell	Experimental Condition*	Ref.
<b>Chemical substances</b>				
Thrombin	↑ PAR1	human endothelial cells	A	93
TNF $\alpha$	↓ PAR1	human endothelial cells	A	124,125
	↑ PAR2, 4	human coronary artery	A	126
IL-1 $\alpha$	↑ PAR2, 4	human endothelial cells	A	127
		human coronary artery	A	126
IL-1 $\beta$	↑ PAR2	human endothelial cells	A	127
TGF- $\beta$	↑ PAR1	human and rat vascular smooth muscle	A	128
PDGF	↑ PAR1	human and rat vascular smooth muscle	A	128
		human endothelial cells	A	127
Lipopolysaccharide	↑ PAR2	rat aortic endothelial cells	B	129
		human endothelial cells	A	127
Phorbol ester	↓ PAR1, 2	human endothelial cells	A	127
Progesterone	↑ PAR1	rat aortic smooth muscle	A+B	130
Dexamethasone	↑ PAR1	rat aortic smooth muscle	A	130
<b>Physical stress</b>				
Balloon injury	↑ PAR1	rat carotid artery	B	60
	↑ PAR2	rat carotid artery	B	80
Shear stress	↑ PAR1 (low shear)	human endothelial cells,	A	131,132
	↓ PAR1 (high shear)	human aortic smooth muscle	A	
Cyclic strain	↑ PAR1	human aortic smooth muscle	A	106
Irradiation	↑ PAR1	rat vascular smooth muscle	B	133
<b>Pathological conditions and disease models</b>				
Atherosclerosis	↑ PAR1	human arterial smooth muscle	B	54,61
Angiotensin II-induced hypertension	↑ PAR1	rat aorta smooth muscle	B	105
Aggregating platelets	↑ PAR1	human and rat vascular smooth muscle	A	128
Preeclampsia	↑ PAR1, 2	human umbilical vein endothelial cells	B	134
Kidney transplantation	↑ PAR1	human endothelial cells	B	135

\*The experimental condition A indicates the observations with the cultured cells or ex vivo experiments, whereas the condition B indicates the observations in vivo.

tissue damage. The vascular effects of proteinases, and consequently the role of PARs, are thus more relevant under pathological conditions.

Thrombin has been shown to either upregulate or downregulate the expression of multiple genes in the cultured endothelial cells.<sup>13,14</sup> The expression of the genes related to angiogenesis and cell growth (vascular endothelial growth factor receptor, angiopoietin, platelet derived growth factors), hemostasis (tissue factor, plasminogen activator inhibitor (PAI)-1), cytokines, and chemokines (interleukins-6 and 8, monocyte chemoattractant protein-1), and cell adhesion (vascular cell adhesion molecule [VCAM]-1, intercellular adhesion molecule-1 [ICAM]-1, E-selectin) have shown to be upregulated by thrombin.<sup>13</sup> These alterations to gene expression may be linked to the phenotypic conversion of endothelial cells to the proinflammatory phenotype. It is possible that such a phenotype conversion observed in the cultured endothelial cells may play a key role in the early stage of vascular

lesion development (Figure). However, this possibility still remains to be elucidated in vivo. In fact, PARs have been suggested to play little role in the inflammatory responses in experimental endotoxemia.<sup>74</sup> Furthermore, the thrombin-induced increase in vascular permeability and angiogenesis<sup>75–78</sup> may also contribute to pathophysiology in vascular lesions (Figure).

### The Role of Smooth Muscle PARs in Vascular Pathophysiology

The expression of PARs, especially PAR1 and PAR2, has been reported to be upregulated in the smooth muscle cells of vascular lesions (Table 3), whereas their expression in the smooth muscle cells of normal arteries is limited.<sup>79</sup> It is thus conceivable that PARs play a more important role under pathological conditions, especially in vascular lesions associated with thrombus formation. Thrombin was not found to induce any contractions in the medial strips of normal rat

aortas, although it induced an increase in the cytosolic  $\text{Ca}^{2+}$  concentrations in cultured smooth muscle cells derived from rat aortas (unpublished observations). In the strips isolated from normal human coronary arteries, the stimulation of PAR1 has been reported to induce endothelium-dependent relaxation, while inducing no contraction.<sup>54</sup> Such endothelium-dependent relaxation was attenuated as the severity of the atherosclerotic lesion increased. In advanced lesions associated with wall thickening, the vasorelaxing response eventually disappeared, while the contractile response then became dominant.<sup>54</sup> In accordance with this report, the expression of PAR1 was found to be localized in the endothelial cells of normal arteries, although it was observed in the smooth muscle cells and macrophages of vessel walls in atherosclerotic lesions.<sup>61</sup> PAR1 and PAR2 have been reported to be upregulated in vascular lesions after balloon angioplasty in rats or baboons, and in human advanced atherosclerotic lesions.<sup>60,61,80</sup> As a result, smooth muscle effects such as contraction, migration, proliferation, hypertrophy, and production of the extracellular matrix become dominant in vascular lesions; contributing to an increase in the vascular tone and the development of vascular lesions (Figure).

### Regulation of the Expression of PARs in Vascular Lesions

Because the expression of PARs in the smooth muscle cells of normal arteries is limited, the upregulation of PARs, especially PAR1 and PAR2, in the smooth muscle cells of vascular lesions is considered to be the key step in contributing to the development of vascular lesions and in increasing vascular tone by these receptors. Elucidating the mechanism that regulates the expression of PARs is thus essential to developing new therapeutic strategies for the prevention and treatment of vascular diseases. Table 3 summarizes the situations which have been reported to be associated with an alteration of the expression of PARs in the vascular system. Chemical substances including cytokines, lysophosphatidic acid, and hormones, and the physical stresses including balloon injury, shear stress, and cyclic strain, have been reported to cause changes in the expression of PARs. On the other hand, atherosclerosis and hypertension have been reported to be associated with the upregulation of PAR1. The molecular mechanisms responsible for changes in the expression of PARs in vascular lesions still remain to be elucidated. In general, the expression of PARs can be regulated at the level of the transcription, translation, membrane trafficking, endocytosis, and lysosomal degradation. Among them, transcriptional regulation appears to play the most important role in the upregulation of PARs in vascular lesions, because upregulation is observed at the mRNA level.<sup>60,61</sup>

The genes for PARs have all been isolated in human DNA.<sup>81–85</sup> The genes for PAR1, PAR2, and PAR3 are clustered on the same chromosome (human 5q13, mouse 13D2),<sup>8,83,84,86–88</sup> whereas the PAR4 gene is located on a different chromosome (human 19p12, mouse 8B3.3).<sup>8,85</sup> The promoter regions of the PAR1 and PAR3 genes were found to be TATA-less, although the consensus binding sequences for some transcription factors were also identified.<sup>81,82,84</sup> The clusters of Sp1-AP1 sites in the PAR1 gene were found to

play an important role in determining basal promoter activity.<sup>81</sup> In vascular endothelial cells, the different isoforms of the Sp family transcription factors were further found to differently contribute to the basal promoter activity of the PAR1 gene.<sup>89</sup> Sp1 has been found to positively regulate transcription, whereas Sp3 inhibits Sp1-mediated transcription in endothelial cells. The reciprocal regulation of transcription of the PAR1 gene by AP2 and Sp1 was also reported in melanoma cells.<sup>90,91</sup> Sp1 positively regulates the transcription of PAR1, whereas AP2 negatively regulates it.<sup>90</sup> In addition, the loss of AP2 expression was found to be correlated to the over-expression of PAR1 and metastatic activity of the melanoma cells.<sup>91</sup> Recently, Kruppel-like transcription factor 2 (KLF2) was identified as a novel regulator of PAR1 expression in endothelial cells.<sup>92</sup> Forced expression of KLF2 suppressed the transcription of PAR1, thereby inhibiting the thrombin-induced accumulation of nuclear factor  $\kappa\text{B}$ .<sup>92</sup>

However, little is known about the intracellular signal transduction regulating the PAR1 transcription. There is one report that has elucidated the involvement of G $\alpha$ i and mitogen-activated protein kinase in the thrombin-induced upregulation of the PAR1 expression in the cultured endothelial cells.<sup>93</sup> Otherwise, the intracellular mechanism for the transcriptional alteration of PARs in vascular diseases still remains largely unclear. The identification of promoter regions, transcription factors, and intracellular signal transduction, all involved in the alteration of PARs expression, will greatly contribute to the understanding of the pathogenesis and development of new strategies for vascular diseases.

### The Possibility of PARs as Therapeutic Targets for Vascular Diseases

The thrombus formation is known to play a critical role in the pathogenesis and pathophysiology of vascular diseases. PARs are considered to be among the most important molecules, which mediate interactions between the coagulation-fibrinolysis system and the vascular walls. Furthermore, the expression of PARs has been found to be upregulated in such vascular lesions as atherosclerosis,<sup>60,61</sup> whereas the development of the proliferative vascular lesions was attenuated in *PAR1*<sup>-/-</sup> mice.<sup>94</sup> As a result, either inhibiting the vascular effects of PARs or preventing the upregulation of PARs in vascular lesions, could provide new therapeutic strategies for the prevention and treatment of vascular diseases. Several PAR1 antagonists with different chemical structures are currently under research and development.<sup>95–98</sup> Some PAR1 antagonists have been reported to prevent the development of vascular restenosis after balloon angioplasty and thrombotic occlusive lesions, in the animal models.<sup>97,99</sup> Further investigation and identification of the involvement of PARs in other vascular diseases would broaden the application of the PAR1 antagonists. A small molecule antagonist for PAR2 has recently been reported,<sup>100</sup> whereas the conventional receptor antagonists for PAR3 or PAR4 have yet to be developed. However, the synthetic peptides corresponding to the third intracellular loop of PAR1, PAR2, and PAR4 have been successfully introduced into the cells as palmitoylated peptides, and they have shown to either mimic the receptor activation or inhibit the intracellular signaling of their respec-

tive receptors, depending on the sequences of the peptides.<sup>101–103</sup> Such peptides having the inhibitory effects could thus be intriguing alternatives for the receptor antagonists.<sup>102,103</sup>

Alternatively, the mechanisms for the regulation of PARs expression could be another focus for the prevention and treatment of vascular diseases. Rac1 has been found to regulate the membrane trafficking of PAR1, thereby regulating the level of PAR1 expression on the cell surface.<sup>104</sup> The inhibition of Rac1 activity using the inhibitors of hydroxy-3-methyl-glutaryl coenzyme A (CoA) reductase (statins), prevented the surface expression of PAR1 and the thrombin-induced elevation of  $[Ca^{2+}]_i$ .<sup>104</sup> Statins could thus potentially be a therapeutic agent preventing the upregulation of PAR1 in vascular lesions. Table 3 suggests other intriguing possibilities for therapeutic agents. For example, angiotensin II may play a critical role in the upregulation of PAR1 in the hypertension model,<sup>105</sup> thus suggesting angiotensin II receptor antagonists could be a therapeutic agent. Oxidative stress is suggested to be involved in the upregulation of PAR1 induced by the cyclin strain, whereas the scavengers of free radicals and inhibitors of NAD(P)H oxidase, have been shown to inhibit the upregulation of PAR1.<sup>106</sup> Thrombin has also been shown to induce its own receptor PAR1 in endothelial cells.<sup>93</sup> PAR1 antagonists may thus be useful not only in inhibiting the vascular effects of thrombin, but also in preventing the upregulation of PAR1. The receptor upregulation is considered to be a key step to the development of vascular lesions and the pathophysiology of vascular diseases. Elucidation of the mechanism of receptor upregulation is thus critical for the development of new strategies for the prevention and treatment of vascular diseases.

### Concluding Remarks

PARs are expressed mainly in endothelial cells under physiological conditions, whereas their expression in smooth muscle cells of normal arteries is limited. The endothelium-dependent regulation of vascular tone may be a major function of PARs in the vascular system under physiological conditions. The agonist proteinases, however, are also activated under such pathological conditions as thrombus formation, hemorrhage, inflammation, and tissue injury. The proinflammatory phenotypic conversion of endothelial cells, as observed in the thrombin-stimulated cultured endothelial cells, may contribute to the early phases of vascular lesion development. Furthermore, PARs, especially PAR1 and PAR2, have been shown to be upregulated in smooth muscle cells under disease conditions. The PAR-mediated effects on smooth muscle cells therefore play a critical role in vascular diseases. The receptor upregulation in smooth muscle cells is thus considered to play a key role in the pathogenesis of vascular diseases. Either inhibiting the effects of PARs or preventing the upregulation of PARs in vascular lesions could be a novel therapeutic strategy for the prevention and treatment of vascular diseases. Elucidating the molecular mechanism for the regulation of PARs expression is thus the next important step in PARs research.

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### Disclosures

None.

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