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Short title: Holinstat Functional dimerization of PAR1 and PAR2

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In this issue, Sevigny and colleagues demonstrate that a protease-activated receptor 1 (PAR1)-PAR2 heterodimer regulates vascular smooth muscle cell (VSMC) hyperplasia following vascular injury [1]. PARs belong to a family of G-protein coupled receptors that are proteolytically activated by a variety of proteases [2, 3]. Cleavage of PARs results in intracellular signaling mediated by activation of various G proteins including $G_{12/13}$, G_q, and G_i [2, 4-6]. The PAR family consists of 4 members, PAR1-PAR4, with PARs 1, 3, and 4 being primarily activated by thrombin, while PAR2 is activated by trypsin and tryptase [2, 3]. PAR1, originally identified as a thrombin receptor on platelets, is widely expressed and has been shown to regulate a multitude of physiological processes including platelet activation [7, 8], regulation of the endothelial cell barrier function [9], and proliferation and de-differentiation of VSMCs [10, 11]. In addition to PAR1, these cells express other PARs [6]. Importantly members of PAR family can physically interact and signal as functional heterodimers in order to regulate cell growth, proliferation and activation [12-15]. PAR1 for example, has been shown to trans-activate PAR2 in human endothelial and COS-7 cells [15], while on the platelet PAR1 may heterodimerize with PAR4 [12].

PAR signaling in the VSMCs following injury results in hyperplasia and de-differentiation, and eventually leads to ischemia and restenosis [11] (see figure). Sevigny and colleagues sought to determine if an interaction between PAR1 and PAR2 is required to mediate these pathophysiological processes in VSMCs following arterial injury [1]. Both receptors were expressed in VSMCs. Interestingly, while thrombin and PAR1 specific agonists increased mitogenesis in these cells, PAR2 stimulation did not. This observation is suggestive of a PAR1-specific regulation of VSMCs following vascular injury with PAR2 signaling alone not playing an essential role. Further supporting the role of PAR1 in VSMC-dependent hyperplasia following injury, activation of PAR1 with the pepducin P1pal-13 resulted in significant thickening of both the medial and intimal areas of the vessel wall, a pathophysiological condition often leading to restenosis. Interestingly, Sevigny and colleagues showed not only that $PAR1^{-/-}$ mice have reduced vessel wall thickening in the presence of a PAR1 agonist, but additionally that P1pal-13 was unable to induce a thickening of either the medial or intimal areas of the vessel wall in the absence of a PAR2 (see figure). Previous observations have shown that PAR2 can mediate signaling through transactivation by PAR1 in human endothelial and COS-7 cells [15] and PAR2 is required for PAI-1 induction by thrombin in murine mammary adenocarcinoma cells [14]. The current study extends this concept to VSMCs and demonstrates that not only can PAR2 work in concert with PAR1 [16] or be transactivated by PAR1 [15], but that the physical presence of PAR2 is required for PAR1 signaling.

The question often presented concerning GPCR dimerization is not whether there is a functional coupling of the receptors but whether this hetero-dimerization is a required component of the signaling process and if a physical interaction of the receptor dimers or oligomers is necessary for signaling to occur. Sevigny and colleagues showed that PAR1 and PAR2 are in the same complex [1] which extend their previous observation that these two receptors reside in close proximity to each other on the cell membrane [16]. Furthermore, PAR1-induced hyperplasia of the medial and intimal areas as well as proliferation of VSMCs were absent in $PAR2^{-/-}$ mice suggesting that homo-dimerization of PAR1 is not able to replace the functional requirement of a PAR1-PAR2 hetero-dimer in PAR1-induced hyperplasia in VSMCs. In addition to hyperplasia, de-differentiation of the VSMCs seems to be also dependent on this hetero-dimer system.

Future studies will need to focus on species-specific differences in the receptor homology and expression within the vessels in order to determine which functional or physical dimerization models best represent the physiological processes occurring in human vessels. To this end,

Sevigny and colleagues point out that species differences in PARs may help explain how PARs regulate VSMCs in vivo [1]. Rat PAR1, for example, might be cleaved at a higher rate by thrombin relative to mouse PAR1 due to differences in the thrombin cleavage site. These differences may be linked to a higher level of restenosis injury and greater sensitivity to inhibition by PAR1 antagonists observed between the rat and mouse models of vascular injury [1]. Determining which model most closely recapitulates human restenosis may allow for the development of novel targets for inhibition of this pathology. Thus, as the authors suggest, even if PAR1 activation kinetics vary, targeting the intracellular loop (which is highly conserved in these species) or PAR2 interaction with PAR1 may represent viable alternatives for preventing VSMCs de-differentiation, hyperplasia and eventual restenosis following vascular injuries.

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None

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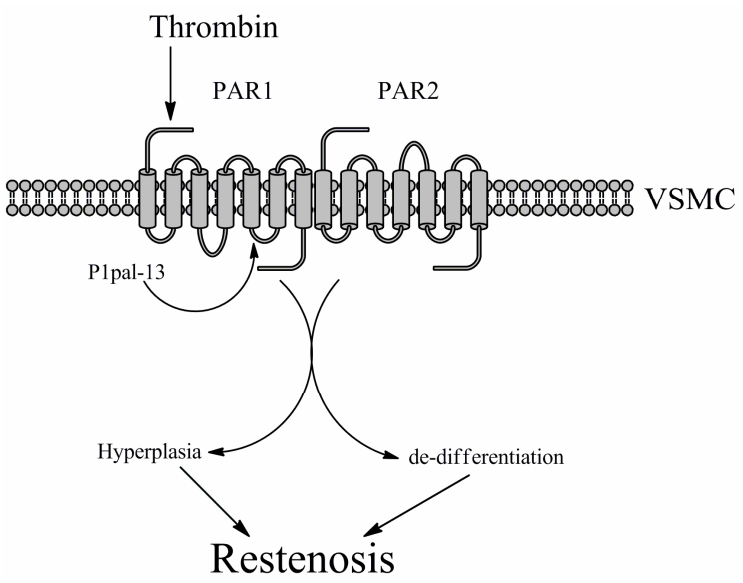
Figure Legend:
Figure. PAR1 and PAR2 are functionally, and likely physically interacting in the vascular smooth muscle cells (VSMCs). PAR1 can be activated either by cleavage of the amino terminal by thrombin (revealing the tethered ligand) or by direct binding of the PAR1 agonist, P1pal-13, to the third intracellular loop of PAR1. PAR1 activation requires the presence of PAR2. Activation of PAR1 in the presence of PAR2 results in VSMCs hyperplasia as well as de-differentiation of the VSMCs both of which play significant roles in the thickening of the vessel wall eventually resulting in restenosis.