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ILLUSTRATED REVIEW

rpth research & practice in thrombosis & haemostasis

Protease-activated receptors: An illustrated review

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Abstract

Proteases are important regulators of cell behavior, survival, and apoptosis. They communicate to cells directly through a special class of G-protein–coupled receptors known as protease-activated receptors (PARs). N-terminal PAR proteolysis unmasks a neo-N-terminus, which serves as a tethered ligand to activate PARs. Using this unique irreversible activation mechanism, PARs relay information across cell membranes. The year 2020 is the 30th year since discovery of the first member of this family, PAR1. In this illustrated review, we highlight achievements in the PAR field over the past 3 decades. Additionally, the known expression profiles of PARs in human tissues and across species are portrayed. We also illustrate the tethered ligand activation mechanism, which is unique to PARs, and PAR regulatory mechanisms. PAR1 was originally named "thrombin receptor" because thrombin was the first protease identified to activate PAR1. However, over the past 30 years, a growing number of proteases have been found to cleave PARs and trigger differential downstream signaling depending on cleavage site, cell type, and species. We exemplify the diversity of PAR1-mediated signaling outcomes in platelets and endothelial cells as pertinent examples to the hemostasis, thrombosis, and vascular biology fields. Further, the termination and regulation of PAR signaling via endocytosis and currently available pharmacologic approaches are depicted. We conclude with portrayal of clinically translational aspects of PAR biology including pharmacologic manipulation and single-nucleotide polymorphisms.

KEYWORDS

antithrombotic therapies, G-protein–coupled receptors, platelets, protease-activated receptors, signaling, thrombosis

Essentials

- Protease-activated receptors (PARs) are G-protein–coupled receptors that mediate protease signaling.
- PARs are expressed widely in the body, and they can be activated by various proteases.
- Cofactors, cellular context, and the activating protease influence downstream signaling.
- PARs are promising therapeutic targets for antiplatelet and antithrombotic therapies.

Abbreviations: ALIX, ALG2-interacting protein X; AP, adaptor protein; APC, activated protein C; ECL, extracellular loop; EPCR, endothelial protein C receptor; ESCRT, endosomal sorting complexes required for transport; GPCR, G-protein–coupled receptor; ICL, intracellular loop; MMP, matrix metalloprotease; PAR, protease-activated receptor; PC, protein C; TM, transmembrane.

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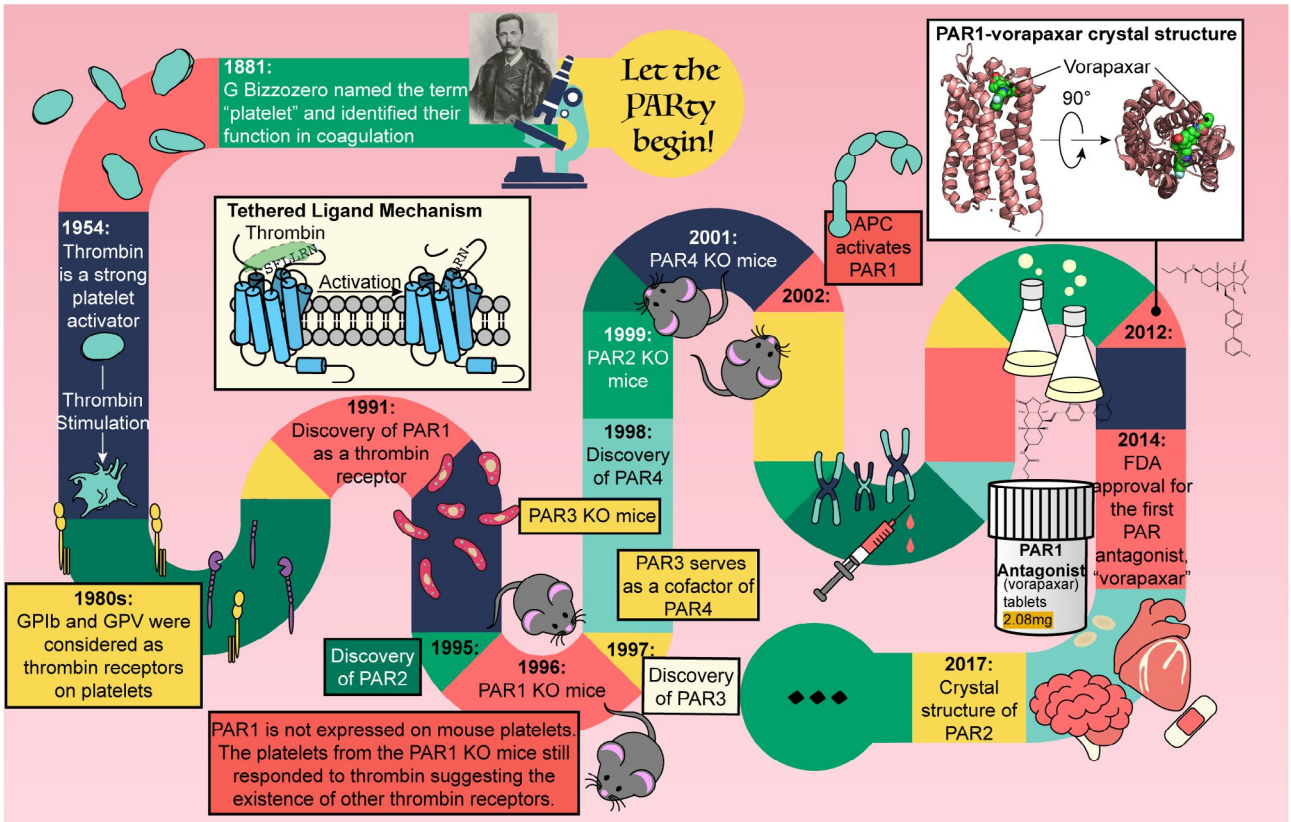
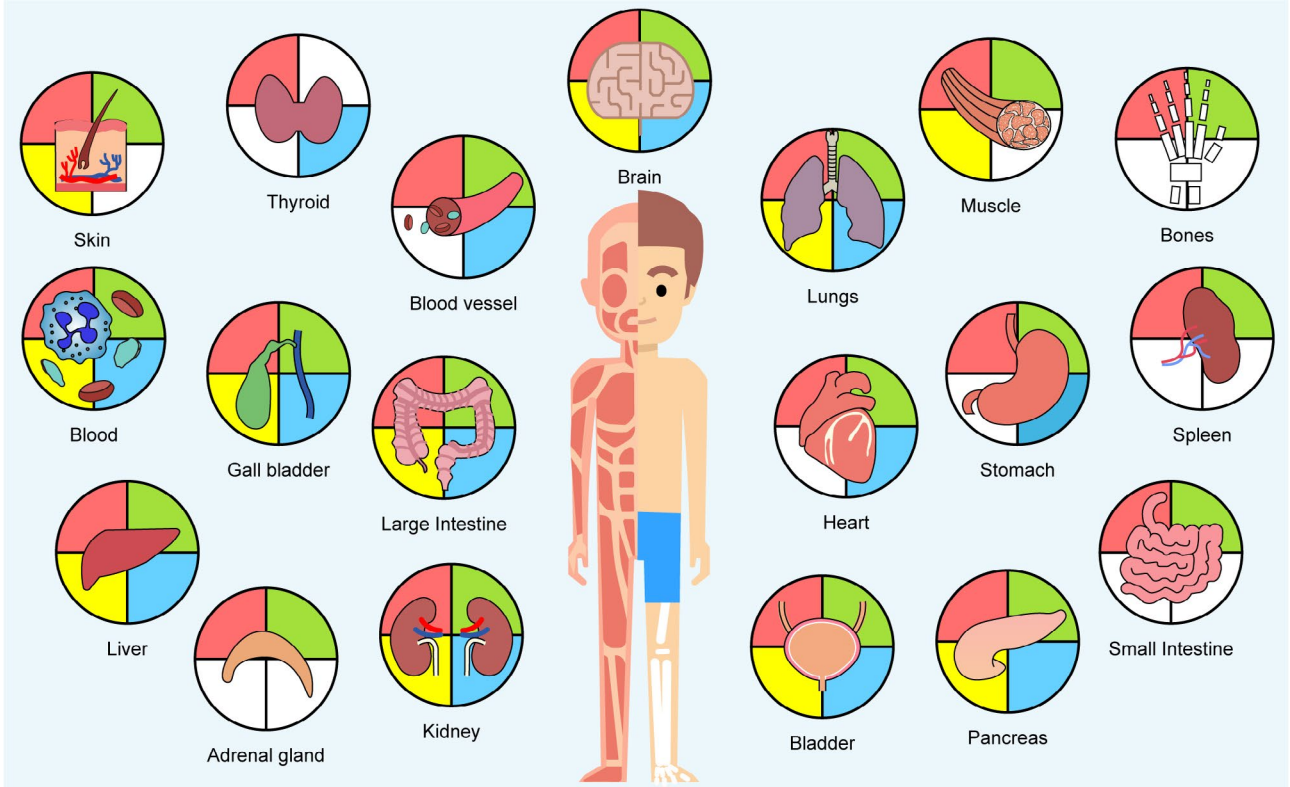
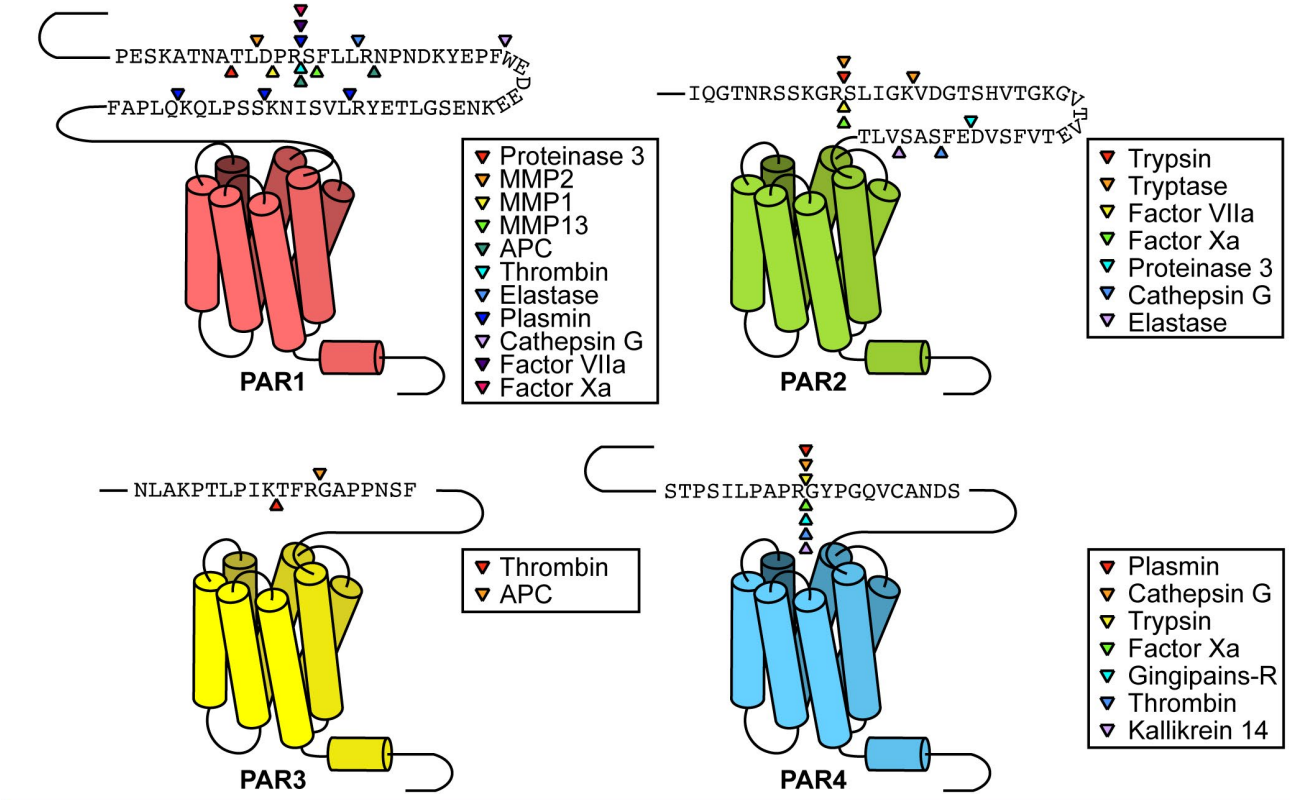


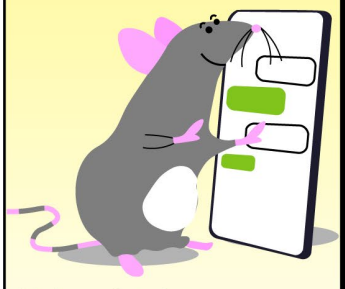
PAR Protein vs Gene Names: PAR1, F2R (Factor II Receptor); PAR2, F2RL1 (Factor II Receptor-Like 1); PAR3, F2RL2 (Factor II Receptor-Like 2); PAR4, F2RL3 (Factor II Receptor-Like 3) [1-3]



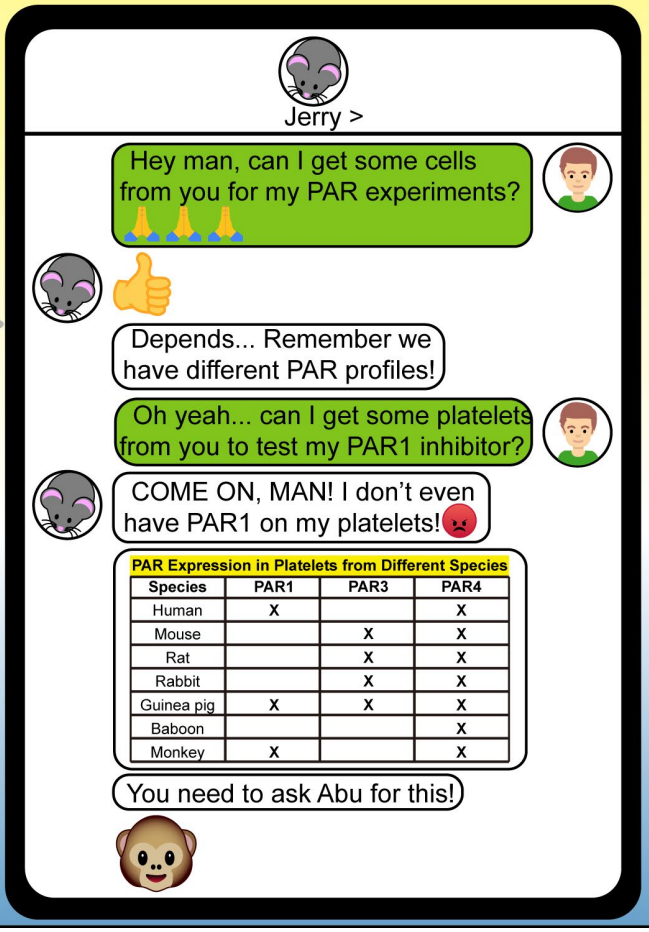
PAR expression as identified by expression analysis or experimental data:
PAR1: red; PAR2: green; PAR3: yellow; PAR4: blue; no known expression: white [4]

Proteases that cleave PARs and their respective cleavage sites [2, 5, 6]



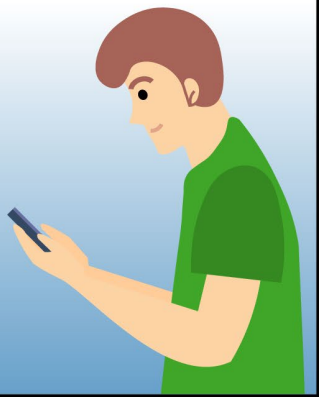


Using platelets as an example, platelet PAR expression profiles vary by species. Human platelets have PAR1 and PAR4. However, mouse platelets express PAR3 and PAR4. Therefore, mice have limited utility as a preclinical model for developing PAR antagonists. Translational studies generally require experiments in non-human primates.

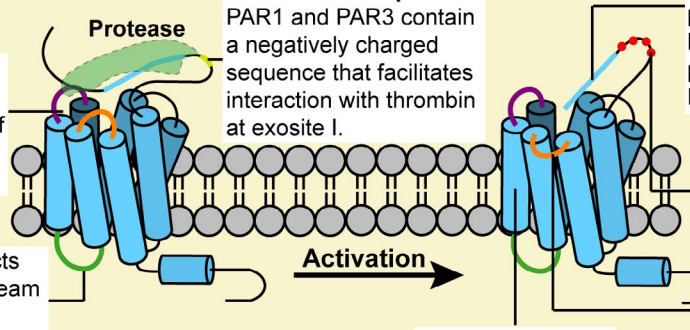


Expression of PARs across Species [2]

Animal models are essential for examining PAR function *in vivo*. However, the PAR expression profiles differ depending on cell type and species.



Tethered Ligand Mediated PAR Activation Mechanism [2, 5]



Tethered Ligand: Proteolysis unmasks the tethered ligand in the N-terminus. Synthesized soluble peptides that mimic the tethered ligand sequence (PAR-activation peptides) can selectively activate PARs independent of cleavage.

Hirudin-like Sequence: PAR1 and PAR3 contain a negatively charged sequence that facilitates interaction with thrombin at exosite I.

ECL2: Critical for tethered ligand-mediated activation of PAR1, PAR2, and PAR4*

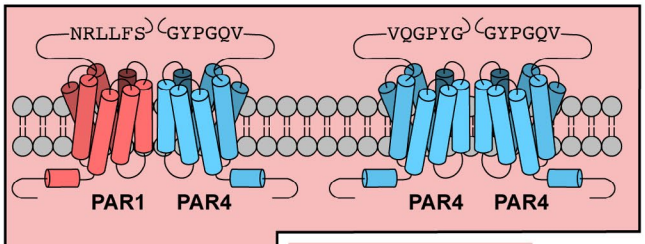
Anionic Cluster: PAR4 uses an anionic cluster to interact with thrombin.

ICL3: Interacts with downstream effectors (G-proteins)

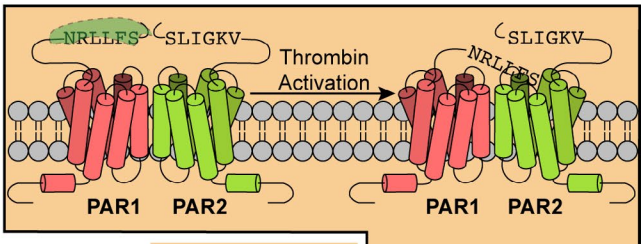
ECL3: Involved in antagonist specificity in PAR1; important for PAR4 activation

TM4 and TM5: Dimer interface of PAR1 and PAR4

*Note: Mouse PAR3 is not known to signal independently whereas independent signaling via human PAR3 remains controversial



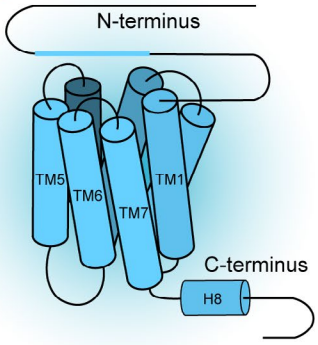
PARs form functional homo- and hetero-dimers with themselves or other PARs to regulate signaling. [2, 5]



PAR1 and PAR2 form hetero-dimers to facilitate transactivation of PAR2 by thrombin-cleaved PAR1. [2, 7]

Homo- and Hetero-Dimers

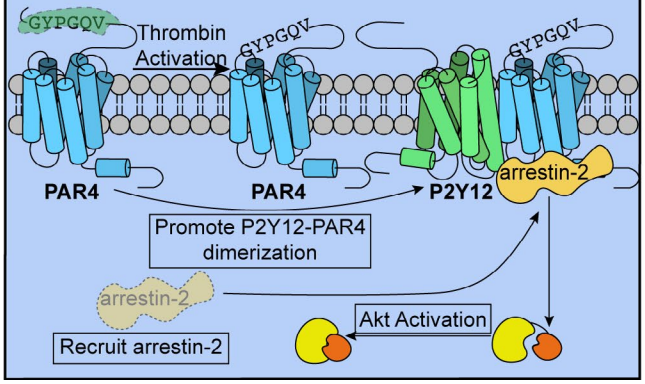
Transactivation



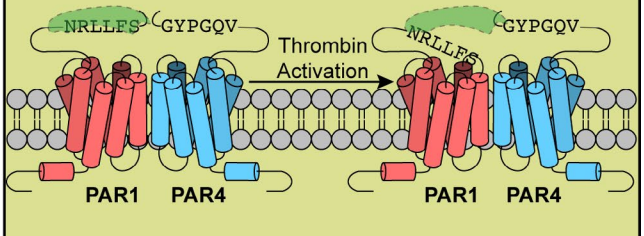
Allosteric Modulation

Cofactor Functions

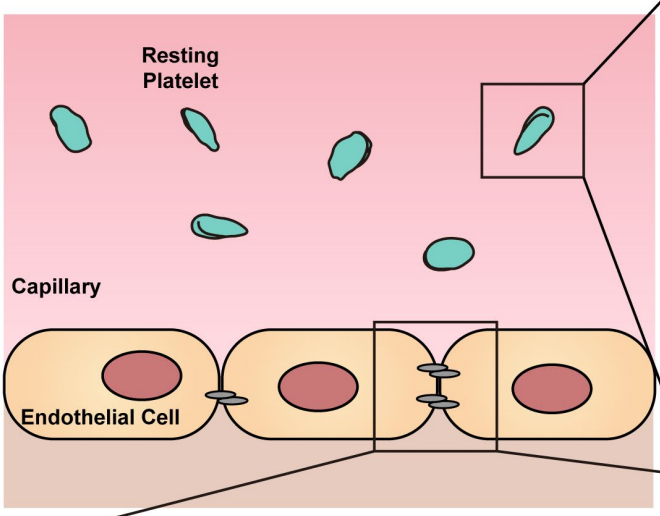
Thrombin-mediated PAR4 activation promotes the formation of P2Y12-PAR4 hetero-dimer, enhances the recruitment of arrestin-2, and supports prolonged Akt signaling. [2, 8, 9]

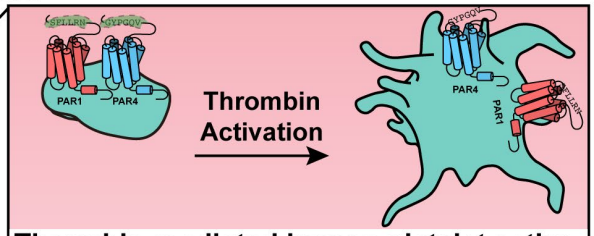


PAR1 serves as a cofactor for thrombin-mediated PAR4 activation. The enhanced rate of PAR4 activation by thrombin is facilitated by the hirudin-like sequence on PAR1. In mouse platelets, PAR3 serves as the cofactor for PAR4 activation. [2, 10, 11]

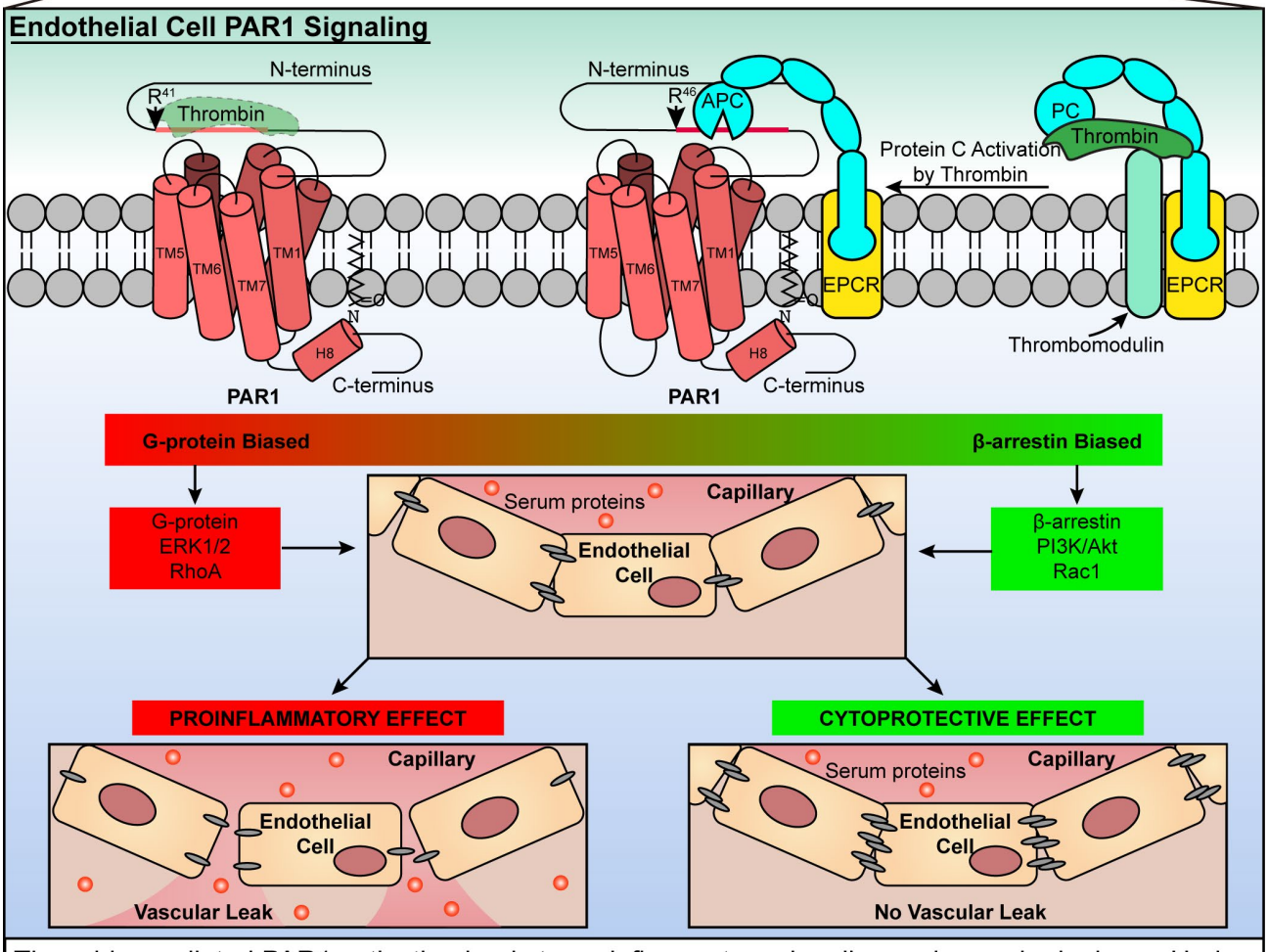


CELLULAR OUTCOMES OF PAR1 BIASED SIGNALING

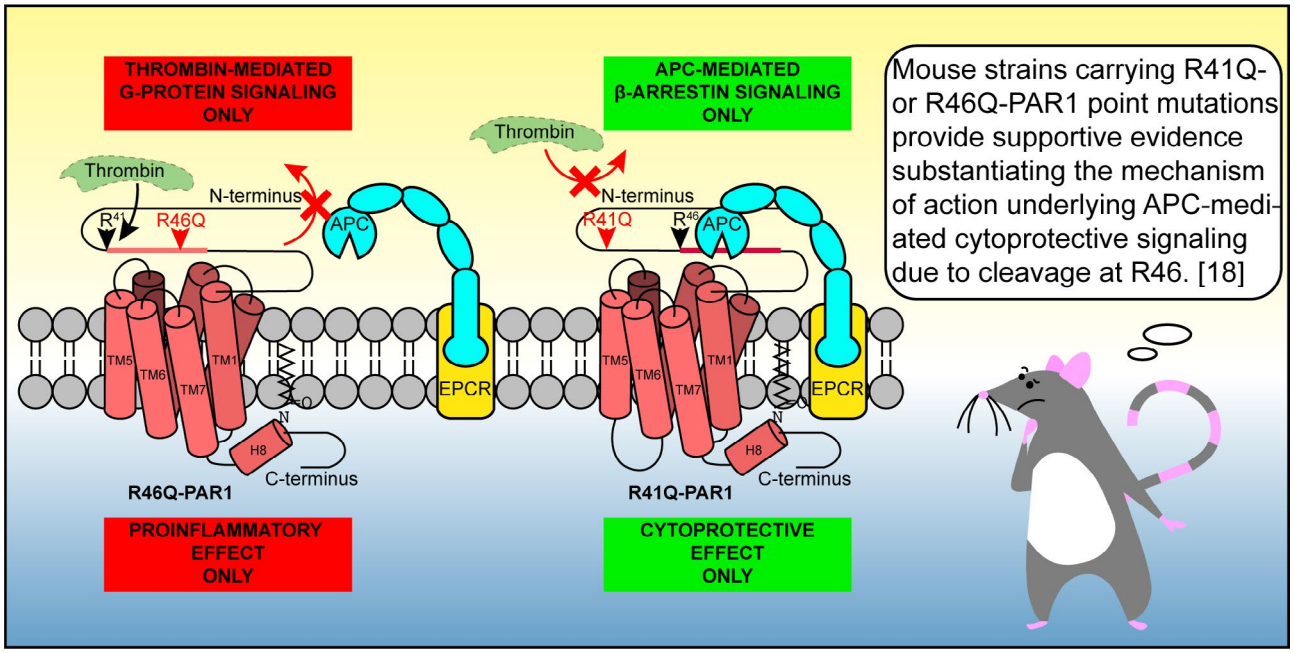




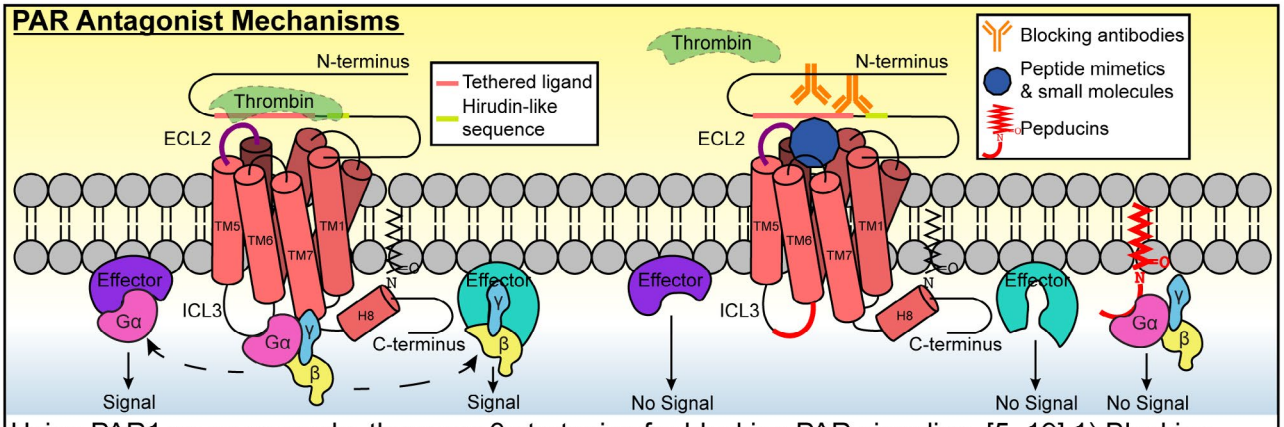
Thrombin-mediated human platelet activation: Thrombin activates PAR1 and PAR4 on human platelets. The PAR1-mediated transient signaling and PAR4-mediated prolonged signaling lead to platelet activation and aggregation. The dual-receptor system covers the full spectrum of physiological thrombin concentrations. [12-14]



Thrombin-mediated PAR1 activation leads to proinflammatory signaling and vascular leakage. Under physiological conditions, endothelial protein C receptor (EPCR) is a critical co-factor that facilitates APC-mediated PAR1 cleavage. In the lipid-raft/caveolae microenvironment, thrombin binds thrombomodulin and activates protein C that is bound to EPCR. This specific microenvironment allows PAR1 to be activated by EPCR-bound APC, but not thrombomodulin-bound thrombin. The EPCR-APC-mediated PAR1 activation triggers cytoprotective signaling and stabilizes the vessel wall. [15-17]

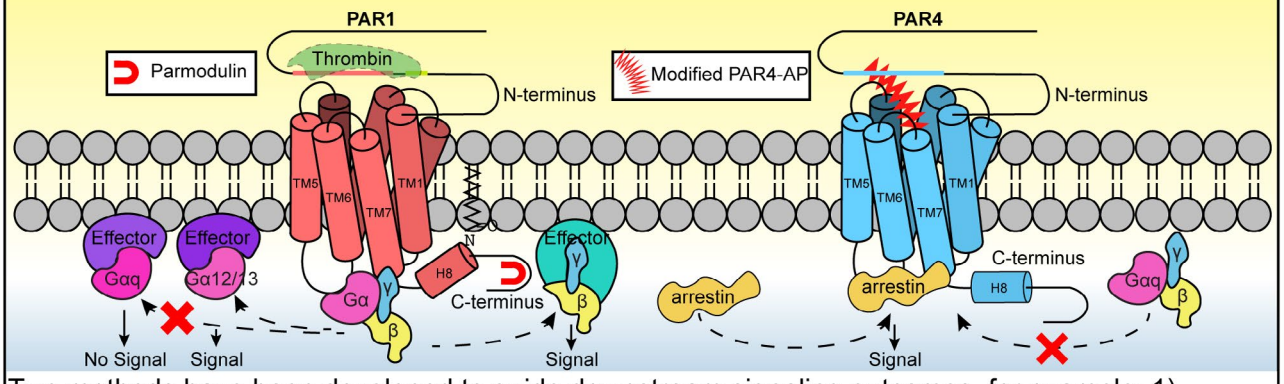


PAR Antagonist Mechanisms

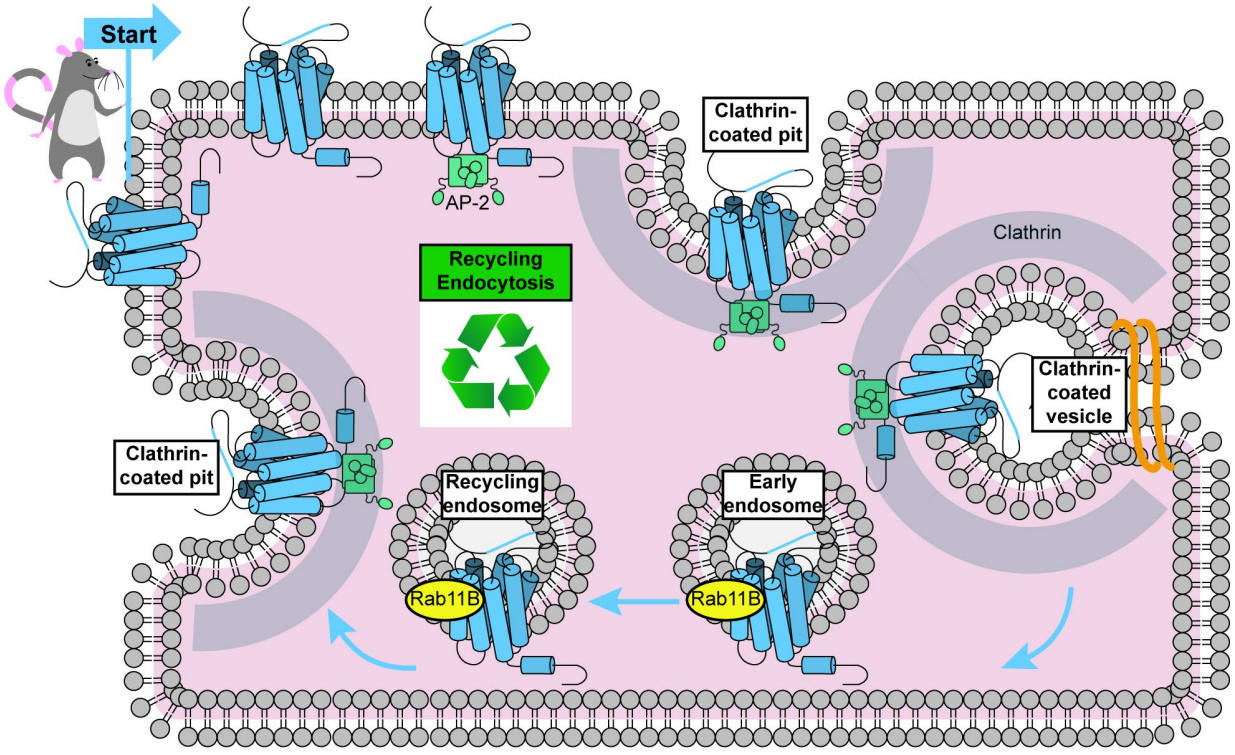


Using PAR1 as an example, there are 3 strategies for blocking PAR signaling. [5, 19] 1) Blocking antibodies prevent protease engagement and cleavage; 2) Peptide mimetics and small molecules block the tethered ligand binding site; and 3) Pepducins act as a decoy to sequester G-proteins away from the activated receptors.

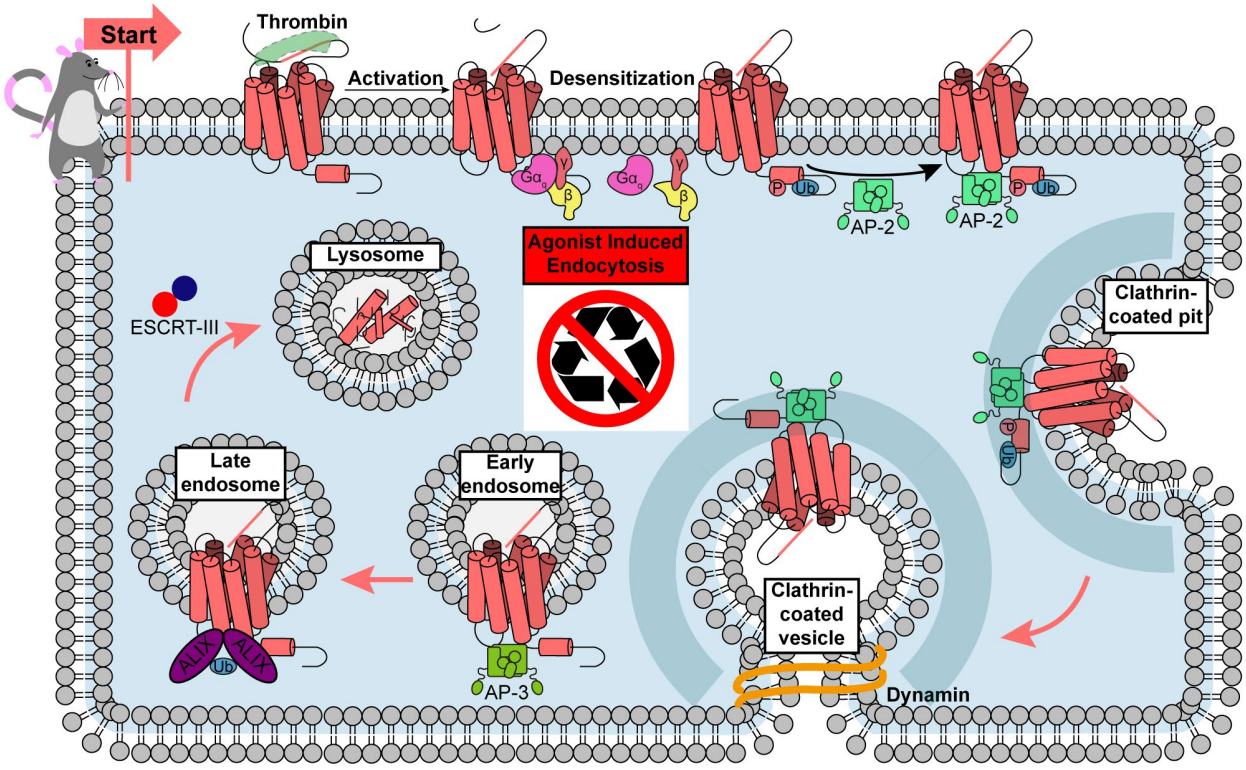
Pharmacological Redirection of PAR Signaling

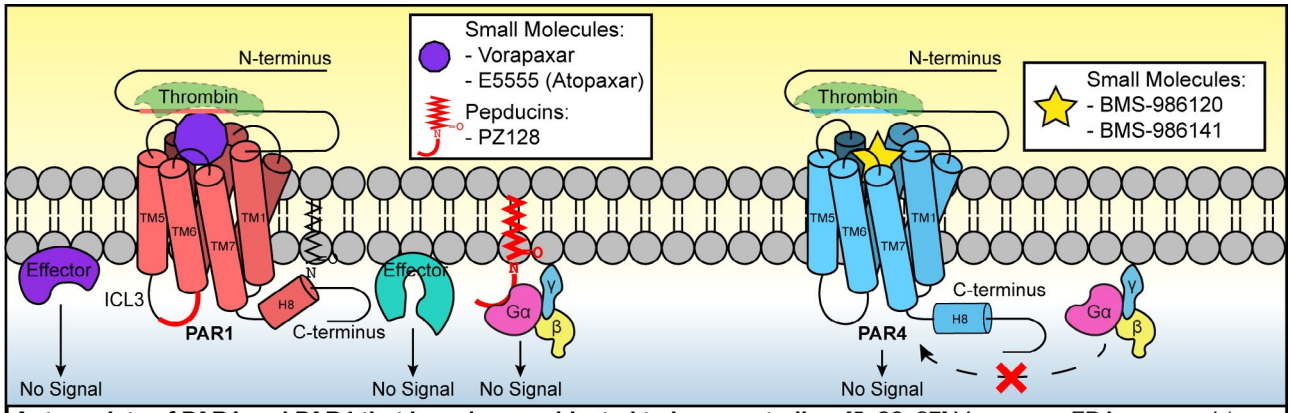


Two methods have been developed to guide downstream signaling outcomes, for example: 1) Parmodulin binds to the PAR1 C-terminus to preferentially engage and activate $G\alpha_{12/13}$ and 2) Modified PAR4-activation peptide (PAR4-AP) biases activated PAR4 towards arrestin engagement as opposed to G-protein signaling. [20-22]

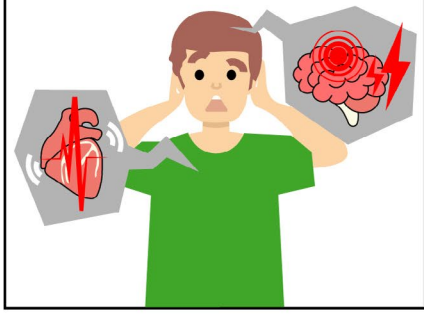


Recycling and Agonist-Induced Endocytic Degradation. Top panel: Constitutive recycling of naïve PARs. The internalization starts with AP-2 binding to PARs, and ends with the recptor recycling back to the cell surface in a Rab11B-dependent manner. Bottom panel: Because cleavage-mediated PAR activation is irreversible, the receptor must be desensitized and eventually degraded to permanently inactivate the signal. AP-2 recognizes activation-dependent phosphorylation and ubiquitination of PARs to initiate receptor internalization. AP-3, ALIX, ESCRT-III are important regulators of endosomal sorting to escort activated-PARs to lysosomes for degradation. [23-25]





Antagonists of PAR1 and PAR4 that have been subjected to human studies. [5, 26, 27] Vorapaxar, FDA approved (see below); E5555 (Phase II Trials; drug development abandoned); PZ128 (completed Phase I studies for coronary heart disease); BMS-986120 (completed Phase I studies on healthy subjects); BMS-986141 (completed Phase II studies for stroke)



Vorapaxar: Target: PAR1 [28, 29]
First in class PAR antagonist to receive FDA-approval (2014)
Indications:
- History of (1) Myocardial Infarction (MI) or (2) Established Peripheral Arterial Disease (PAD)

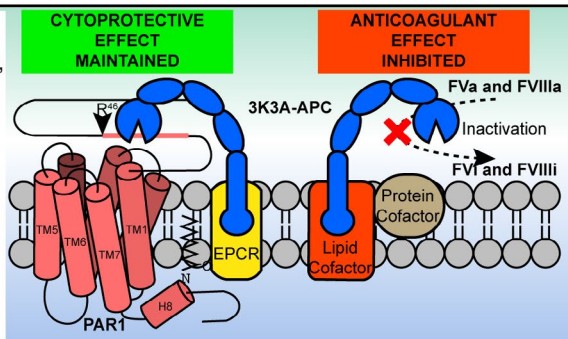
Contraindications and Black Box Warning:
- History of (1) Stroke, (2) Transient Ischemic Attack (TIA), (3) Intracranial Hemorrhage (ICH), or (4) Active Bleeding
- Antiplatelet agents, including vorapaxar, increase the risk of bleeding, including ICH and fatal bleeding.

Dose: 2.08 mg daily in combination with aspirin and/or clopidogrel.
Due to its very long half-life, vorapaxar is effectively an irreversible platelet antagonist.

Clinical Trials of 3K3A-APC [30, 31]

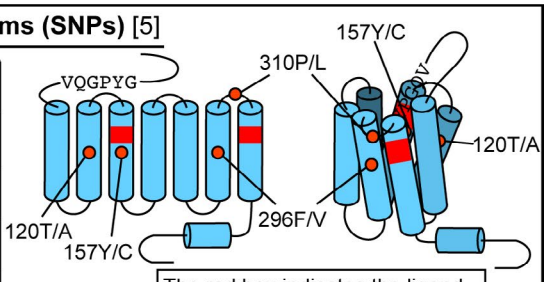
APC is a serine protease that has antithrombotic, antiinflammatory, antiapoptotic, and cytoprotective effects. Previous clinical studies of recombinant APC were abandoned due to increased bleeding risk. This molecularly engineered recombinant APC variant (3K3A-APC) retains its PAR1-mediated cytoprotective signaling function but retains <10% of its anticoagulant activity.

The NeuroNEXT consortium conducted a Phase II study of 3K3A-APC for ischemic stroke which was completed in 2018 and demonstrated neuroprotective effects with a trend toward reduced intraparenchymal hemorrhage.



PAR4 reactivity is affected by single nucleotide polymorphisms (SNPs) [5]

SNP	Residue	Alleles	Location	Effect	Potential Mechanism
rs773902*	120	Thr→Ala	TM2	↓ reactivity	Close to ligand binding site
	157	Tyr→Cys	TM3	↓ reactivity	Affects receptor transportation
rs2227346	296	Phe→Val	TM6	↓ reactivity	Affects ligand binding/ Na^+ pocket microswitch
rs2227376	310	Pro→Leu	ECL3	↓ reactivity	Affects ECL3 rigidity



* The minor allele frequency (MAF) for the Thr^{120} is 0.19 in individuals with European ancestry and 0.45 for those with African ancestry

PARting Conclusions: PARs have a broad tissue expression profile and flexible downstream signaling outcomes, suggesting important roles in (patho)-physiologies beyond hemostasis and vascular biology that may impact multiple organ systems. Thus PAR-directed pharmacotherapeutics may hold promise in variety of diseases, but the widespread tissue expression of PARs may lead to unintended toxicities that need to be considered in preclinical models and clinical trials.

ACKNOWLEDGMENTS

The authors regret that work from some colleagues could not be referenced or discussed due to space limitations, in particular, the experimental works identifying tissue expression of PARs. The authors would thank Maria de la Fuente, Elizabeth Knauss, and Amanda Waller for critical suggestions and helpful discussion.

RELATIONSHIP DISCLOSURE

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

XH, MN, and BAK wrote the manuscript.

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and tolerability of 3K3A-APC, a recombinant variant of human activated protein C, in combination with tissue plasminogen activator, mechanical thrombectomy or both in moderate to severe acute ischemic stroke. Ann Neurol. 2019;85(1):125–36.
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