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Research Highlight

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Apelin/APJ system: a novel promising therapy target for thrombotic diseases

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APJ, which was initially identified as a gene with closest homology to the angiotensin II type 1 receptor, is a seven transmembrane G protein-coupled receptor. Apelin is an endogenous ligand of the APJ originally isolated from bovine stomach extracts. There are several isoforms of apelin. Apelin preproproteins contain 77 amino-acid residues, which can be cleaved to form shorter bioactive isoforms, including apelin-36, apelin-17, apelin-13, apelin-12, and so on. Apelin/APJ receptor is extensively distributed in the central nervous system and peripheral tissues. Apelin/APJ system involves in a wide range of physiological and pathological functions. For example, apelin lowers blood pressure via a NO/cGMP-dependent mechanism. Apelin-13 maintains the Ca^{2+} transient against ischemia/reperfusion in cardiomyocytes. Furthermore, apelin-13 promotes cell proliferation and angiogenesis via PI3K/AKT activation [1,2].

It is well known that NO/cGMP and Ca^{2+} may serve as important mechanisms to adjust platelet activation. Platelets are the smallest elements of the blood. Platelets are anucleate cells with a variety of intracellular organelles, including different types of secretory granules. Physiologically, platelets mainly participate in hemostasis to prevent excessive blood loss. However, abnormalities in platelet function, such as thrombosis or bleeding, usually result in severe and lethal consequences. For example, platelet aggregation contributes to many thrombotic diseases, including peripheral artery disease (PAD), acute coronary syndrome (ACS), myocardial infarction (MI), heart attacks (HA), strokes, arteriosclerosis (AS), and cancers. Several specific stimuli including thrombin, ADP, and elements of the extracellular matrix initiate the activation of platelets and then lead to platelet aggregation [3]. Although several antiplatelet drugs which mainly target at cyclooxygenase-1, purinoceptor 12 (P2Y12)-receptor, and integrin αIIbβ3 are currently used to treat for thrombotic complications, a large number of patients continue to show adverse thrombotic events. The main reason is that the current treatment is unable to block multiple platelet signaling pathways. It is necessary to develop novel antithrombotic agents which can contribute to the crosstalk of platelets.

How about the role of apelin/APJ in the regulation of platelets? Adam *et al.* [4] recently reported that apelin/APJ receptor is expressed in human and mouse platelets. Apelin-13 inhibits a low concentration of thrombin- or collagen-induced platelet aggregation in human and mouse. Furthermore, they found that apelin$^{-/-}$ mice displayed a prothrombotic phenotype and presented unstable hemostasis with shorter bleeding time. Platelets separated from apelin$^{-/-}$ mice displayed increased aggregation after stimulation with low concentrations of thrombin or collagen, compared with wild-type platelets. Intravenously injected apelin-13 prolonged bleeding time and prevented thrombosis in mice. These data indicated that apelin-13 is directly involved in the regulation of platelet activation. Furthermore, Adam *et al.* [4] also explored the antiplatelet mechanism of apelin-13 in detail. First, platelets stimulated with agonists were found to result in transient activation of integrins. Apelin-13 inhibits the adhesion of resting and thrombin-activated platelets to fibrinogen, which is dependent on integrin αIIbβ3. Second, the elevation in calcium was found to be a crucial event in platelet activation. Apelin-13 plays the obvious role in inhibiting calcium mobilization via the PI3K pathway. Third, some signaling mechanisms including cAMP, cGMP, and TXA2 synthesis were found to participate in platelet activation. Apelin-13 fails to alter cAMP accumulation but stimulates cGMP production via the NO pathway. Apelin-13 also has an obvious inhibitory effect on TXA2 synthesis. Therefore, we can conclude that the antiplatelet mechanisms of apelin-13 involve integrin αIIbβ3, calcium mobilization, cGMP production, and TXA2 synthesis (**Fig. 1A**).

Abnormalities in platelet function are related to many thrombotic diseases, such as PAD, ACS, MI, HA, strokes, AS, and cancers. A large number of studies have shown that apelin/APJ may be involved in some of those thrombotic diseases. Acute apelin administration in humans leads to peripheral and coronary vasodilatation. Apelin plays a protective role in the MI. It has been proved that apelin induces cell proliferation and tube formation in endothelial cells.

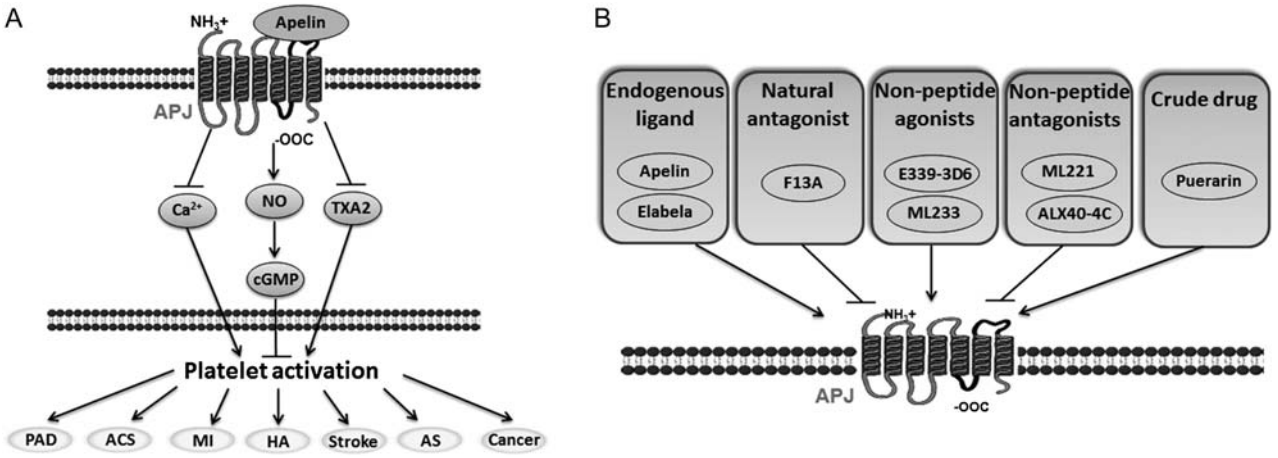

Figure 1. The antiplatelet mechanisms of apelin-13 involve integrin αIIbβ3, calcium mobilization, cGMP production, and TXA2 synthesis (A) Apelin/APJ signaling pathway may be a novel therapy target for thrombotic diseases, including PAD, ACS, MI, HA, strokes, AS, and cancers. (B) Drugs targeting at apelin/APJ include endogenous ligand apelin, elabela, natural antagonist F13A, non-peptide agonists E339-3D6 and ML233, non-peptide antagonists ML221 and ALX40-4C, and crude drug puerarin.

Apelin level is reduced soon after MI, but exogenous apelin has beneficial effects on blood vessel formation, which contributes to the alleviation of MI [5]. In ischemia stroke, apelin-13 protects against cell apoptosis by activating AMP-activated protein kinase pathway [6]. Apelin-13 also plays a neuroprotective role in the experimental ischemic stroke by inhibition of inflammation. Serum apelin levels were found to be significantly higher in metabolic syndrome patients than in normal controls, and associated with coronary atherosclerosis. Abnormal platelet may be related to lung cancer. The platelet distribution width value was found to be significantly higher in the lung cancer group than in the normal control group; however, the values for plateletcrit and mean platelet volume are lower in the lung cancer group. Apelin can also promote lung adenocarcinoma cell proliferation [7]. Apelin/APJ signaling pathway may be a novel therapy target for treating lung cancer by regulating the function of platelets.

At present, the main drugs for recurrent thrombotic diseases are aspirin and clopidogrel which can inhibit platelet activation induced by TXA2 and P2Y12 (ADP receptor), respectively [8]. However, thrombotic diseases still occur even under the treatment with these antiplatelet drugs. This can be explained by the existence of multiple pathways contributing to platelet activation and aggregation that are not inhibited by aspirin or P2Y12 antagonists, as in the case of thrombin-mediated pathways. Adam *et al.* [4] demonstrated that apelin/APJ receptor inhibits thrombin- and collagen-induced platelet activation and aggregation, which reveals the new biological function of the apelin/APJ system. Apelin-13 plays antiplatelet roles mainly under the influence of integrin αIIbβ3, calcium mobilization, cGMP production, and TXA2 synthesis. Apelin, which plays an important role in the crosstalk of platelets, may be a more effective antithrombotic agent than the currently used drugs. In addition, apelin is a naturally occurring inhibitor of platelet activation compared with other drugs such as aspirin and clopidogrel. Therefore, apelin or its derivatives may be used for the prevention of thrombotic or hemorrhagic diseases with high efficiency.

Recent studies suggested that the apelin/APJ system may play an important role in the process of thrombotic diseases. Therefore, the apelin/APJ system may become new drug targets [9], and the drugs targeting at apelin/APJ may potentially be developed to treat the relevant diseases. Apelin is the endogenous ligand of APJ. However, apelin is not the only endogenous ligand of APJ. Recently, another peptide elabela has also been found to be the endogenous ligand of APJ. Elabela can also increase cardiac contractility and induce coronary vasodilation, which is similar to apelin. Besides these two peptide ligands, E339-3D6 and ML233, are the non-peptide agonists of APJ, while F13A is the natural antagonist isoform of APJ [9]. However, ML221 and ALX40-4C are the antagonists to APJ receptor [9]. Crude drug puerarin may also target at APJ receptor [9].

In summary, drugs targeting at APJ and apelin may become new theraputics for thrombotic diseases by influencing the integrin αIIbβ3, calcium mobilization, cGMP production, and TXA2 synthesis (**Fig. 1B**). Although the successful development of those drugs will require further research, apelin/APJ receptor should be considered as a potential therapeutic target for thrombotic diseases.

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