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Research Highlight

Unanticipated role of apelin: regulation of miRNA generation

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Recently, a paper by Kim *et al*. [1] in Nature Medicine magazine in January, 2013 showed that apelin (also known as APLN) inhibits fibroblast growth factor 2 (FGF2) and FGF receptor 1 (FGFR1) expression to ameliorate pulmonary hypertension by regulating the expression of miR-424 and miR-503. This study revealed the molecular mechanism of apelin in inhibiting the process of pulmonary arterial hypertension (PAH) and discovered the role of apelin in regulating miRNA generation for the first time. miRNA functions in the transcriptional regulation of gene expression to control cellular processes. miRNA is a key regulatory factor of protein expression, but the generation and regulation mechanism of miRNA is still unclear. These novel findings bring us inspiration for further research, especially on the mechanism of miRNA generation. Experiments on revealing endogenous active substance, which regulates the generation of miRNAs or revealing miRNAs that regulate the expression of apelin, may bring more breakthroughs in the future.

PAH is characterized by vascular remodeling associated with obliteration of pulmonary arterioles and formation of plexiform lesions composed of hyperproliferative endothelial and vascular smooth muscle cells. Recent studies have suggested that apelin is a novel PAH endothelial function homeostasis-related factor. Alastalo *et al*. [2] found that apelin expression is decreased in endothelial cells of the pulmonary hypertension. However, the exact mechanism remains poorly understood. FGF2 is highly expressed in PAH and plays an important role in the progress of PAH by promoting proliferation [3] and inhibiting apoptosis [4] in endothelial cells and smooth muscle cells. miRNA is a fundamental factor of numerous cellular events by regulating RNA modification, transcription, and translation. Current studies of miRNA showed its critical function in the development of PAH. Morphological changes of plexiform vasculopathy in the end-stage PAH lung are reflected by alterations at the miRNA level [5]. Kim *et al*. [1] integrated these isolated observations into a mechanism and identified the miRNA-FGF signaling axis that is apelin-dependent in the maintenance of pulmonary vascular homeostasis. Previous studies found that hypoxia induces endothelial function injury. Accumulating evidence revealed that hypoxia also induces the expression of apelin [6,7], which is reduced in PAH. Actually, apelin expression and secretion, which are strongly induced under hypoxic conditions, are the early response [7]. Mechanisms that maintain sustained expression of apelin may contribute to preventing injuries caused by hypoxia and restoring the function of endothelial cells in PAH. These findings supported the development of novel therapeutic strategies to augment apelin, as well as to inhibit FGF2 signaling. In portal vein hypertension, another vascular disease, apelin/APJ presents a novel therapeutic target. APJ antagonist F13A effectively decreased the formation of portosystemic collateral vessels [8]. In atherosclerosis (AS), increasing evidence tends to prove that apelin is a novel therapeutic target for AS [9]. Although no effective treatment for PAH is available at present, certain medicines are available to mitigate disease progression. Considering its protective effect on vasodilatory and endothelial cells, apelin may be a more efficacious target for PAH therapy.

In past decades, apelin was suggested to involve in numerous physiological processes, including vasodilation, systole, salt and water balance, as well as pathophysiological processes such as high blood pressure, cancer, and so on. There must be many other undiscovered functions of apelin. The findings of the intimate relationship between apelin and miRNA provide much fresh thinking for the study of apelin. The study on miR-424 and miR-503 will help to discover additional features of apelin. Park *et al*. [10] found that the high expression of miR-424 and miR-503 is significantly implicated in chemoresistance and tumor progression in ovarian cancer, which probably regulates cancer stem cell processes. These results indicated that apelin probably plays a vital role in epithelial mesenchymal transition in cancers. A recent study revealed that miR-503 makes women predispose to lupus [11]. The relationship between apelin and lupus has not been explored. More evidence is required to confirm whether apelin controls the effects of miR-424 and miR-503 in the above process. There are some controversial reports of apelin/APJ effects on AS. Chun *et al*. [12] found that apelin decreases AS formation by blocking AngII actions in ApoE-KO mice. But Hashimoto *et al*. [13]

demonstrated that apelin/APJ system is the mediator of oxidative stress-linked AS in vascular tissue. Although apelin-13 is possibly a novel risk factor for AS occurrence and development by promoting vascular smooth muscle cell proliferation [14] and monocytes-endothelial cell adhesion [15], the mechanism of apelin on AS are still unclear. Further experiments are necessary to clarify the effect of apelin on AS, and miRNA is an excellent research strategy [16]. Gene ontology annotation analysis on conservation targets of miRNA-424 and miRNA-503 can be used to predict other roles of apelin. Furthermore, whether some other miRNAs can be regulated by apelin remains to be answered. As apelin can regulate the expression of miR-424 and miR-503, it tells us that other cytokines may play the same role. The homology domain or similar functional factor compared with apelin might possess the effect on the regulation of miR-424 and miRNA-503 expressions. Since miRNA is closely related to apelin, there should be one or more miRNA(s) regulating the expression of apelin. Database in the online server (http://www.targetscan.org/) was used to find possible miRNAs that regulate the transcription of apelin and APJ. Results showed that miR-182, miR-124/124ab/506, miR-15abc/16/16abc/195/322/424/497/190, miR-503, or miR-137/137ab might be the regulators of apelin expression by combining with its 3′-untranslted region. More examinations are necessary to determine whether the predicted miRNA regulates apelin secretion or not.

While the roles of miRNAs have been extensively studied, the regulatory mechanism of miRNA expression is rarely reported. Manavella *et al*. [17] found that CPL1 is a crucial element in the miRNA biogenesis in plant (thale cress). CPL1 destroys miRNA generation by hyponastic leaves 1 (HYL1, one of RNA binding proteins) dephosphorylation. Kim *et al*. [1] first confirmed that apelin regulates miRNA expression in homo cells. Now, the question is how apelin affects the expression of miR-424 and miR-503. Here, we propose that miR-424 and miR-503 are the intron siRNA of *apelin* gene or apelin specifically inhibits the expression and/or function of the trans-acting factors of miR-424 and miR-503. Consequently, apelin can only regulate the generation of miR-424 and miR-503. Otherwise, apelin may regulate the expression of RNA polymerase or transcription factors to control the generation of miR-424/miR-503. If so, apelin could control many other miRNAs transcription. The discovery that apelin regulates miRNA generation opens opportunities for the development of miRNA research. Additional studies will contribute to reveal the mechanism of miRNA generation.

Here, we noticed that apelin expression is significantly decreased in pulmonary artery endothelial cell lines from PAH patients. Real-time quantitative PCR was used to detect the *apelin* mRNA expression, and western blot analysis was used to detect the pre-pro apelin. As cleaved active forms of apelin exist in human body, the study on the role of specific isoforms in the vasculature would be important. Therefore, measuring the apelin isoform by enzyme-linked immunosorbent assay is necessary for PAH clinical treatment. Anyway, the work of Kim *et al*. [1] opens more areas for further exploration. The investigations on the function of apelin as well as the isoforms and miRNA will provide potential effective targets for diseases treatment.

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