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Review Article

Inhibition of Aortic Medial Calcification: miPEP-200b and miRNA-200b as Potential Mediators

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Abstract

When considering the flow of genetic information in a cell, the traditional pathway of DNA to RNA to protein is what first comes to mind. In recent years, it has been clearly demonstrated that this pathway must be revised from new data and discoveries. The extensive study of noncoding RNA (ncRNA) has led to the discovery of many of its functions that were once unknown. Perhaps even more intriguing is our recent discovery that was made in an already new field of study. We demonstrated that primary microRNA-200a (pri-miRNA-200a) and pri-miRNA-200b possess open reading frames (ORF) that were recognized by ribosomes, allowing the pri-miRNAs to be translated into two peptides, miPEP-200a and miPEP-200b. Furthermore, studies have shown that these peptides are involved in the inhibition of cell migration in breast and prostate cancer cells and may even serve as significant prognostic markers of clinical outcomes. We have previously shown that miPEPs have downstream functional effects very similar to their miRNA counterparts, resembling many other protective mechanisms observed in nature. This "double-edged functional sword" allows for continued activity despite decreased functionality in one part of the system. Although the anti-neoplastic role of these peptides has recently been an area of interest, not much research has been published regarding their role in cardiovascular disease. In one study, it was demonstrated that a single nucleotide polymorphism in the gene coding for miRNA-200b might result in increased protein kinase A (PKA) activity that ultimately leads to activation of thrombocytes and ensuing atherosclerosis. PKA is not only involved in platelet activity but is rather known to play a role in a multitude of cellular pathways. Of interest is PKA's involvement in medial aortic calcification, a process that has been implicated in isolated systolic hypertension (ISH); this condition is associated with increasing age. We hypothesize that in the same way that miRNA-200b plays a role in decreasing PKA activity in atherosclerotic processes, the peptide miPEP-200b may also act as an inhibitor of PKA-induced aortic medial calcification. If this association is shown to be present, focused therapy with miPEP-200b and miRNA-200b, along with PKA inhibitors, may significantly reduce the incidence, as well as prevalence, of isolated systolic hypertension in older age groups, leading to a decreased incidence of diastolic heart failure secondary to longstanding hypertension.

Introduction

In molecular biology, the established central dogma is the widely accepted framework of genetic information flow. This process begins with the DNA transcription from the nucleus of the cell into linear, single-stranded messenger RNA (mRNA). Ribosomes then read the mRNA strand following transportation to the cytoplasm, and translation of the code into single amino acids, which are added sequentially to the growing peptide. Once the peptide is fully synthesized, both the mRNA strand and the peptide are released from the ribosome [1]. Although this pathway has been established universally as the only way for protein synthesis, only 1.5% - 2.5% of the human genome codes for proteins [2]. The remainder of the genome produces noncoding RNAs (ncRNAs). These ncRNAs can be further classified into various subtypes; of note are miRNAs, small nucleolar RNAs (snoRNAs), long noncoding RNA (lncRNA), and circular RNA (circRNA) [3]. Although ncRNAs have always been thought to have no role in protein synthesis, many previously unknown indirect functions of ncRNA have been elucidated within the past five years. They have been shown to be involved in many settings such as macrophage activation during an immune response, abnormal expression in diabetic wounds, retinal diseases, and even endometrial physiology and disease states such as endometriosis [4-7]. Perhaps the most groundbreaking findings in the realm of ncRNAs have been their association with various human cancers [8]. Most research has been focused on exploring the epigenetics and post-transcriptional activity of ncRNA, which allows these molecules to exert regulatory effects on the expression

of the genome [9]. By directly binding to mRNA strands, miRNAs are able to regulate their ability to eventually lead to protein synthesis [10]. It has been demonstrated that this process is extremely precise, allowing targeting of mRNA with high specificity. Interestingly, the miRNA family has identical 5' regions with the 3' region differentiating these molecules and their specificities [11]. Although protein translation and miRNAs have conventionally been thought to have such an indirect relationship, our recent discoveries suggest a much more direct engagement between the two. We have shown that the sequences in certain pri-miRNAs, more specifically pri-miR-200a and pri-miR-200b, contain ORFs that are able to be recognized by ribosomes and subsequently translated directly into protein products, miPEP-200a and miPEP-200b. Even more intriguing is the potential role of these pri-miRNA-derived peptides (miPEPs) in cancer repression [12-15].

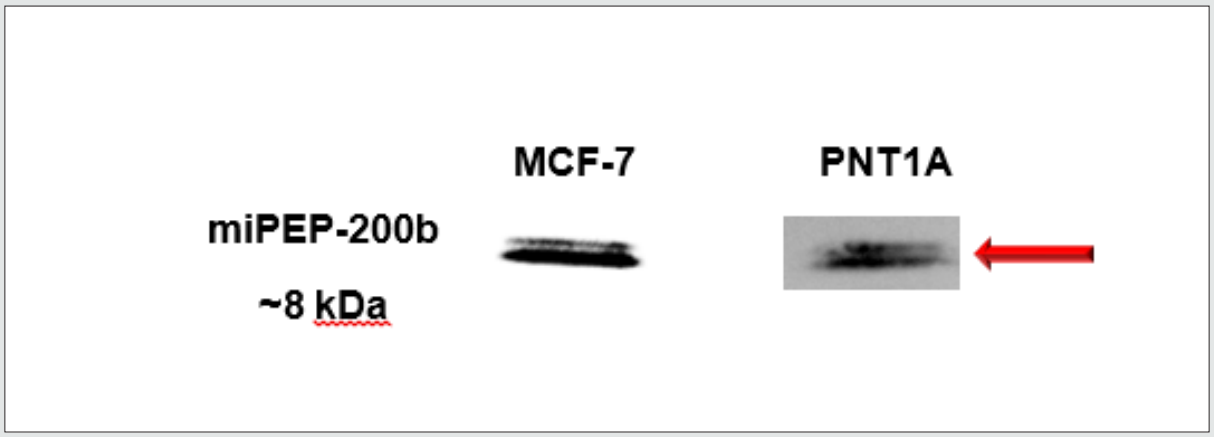

Figure 1: Expression of miPEP-200b in prostate cells (PNT1A) and breast cells (MCF-7). MCF-7 and PNT1A cells were harvested and prepared for western blot. Total extracts were subjected to western blot analysis using miPEP-200b (antibody raised against mi-PEP-200b). 8 Kda band represents expected band for miPEP-200b.

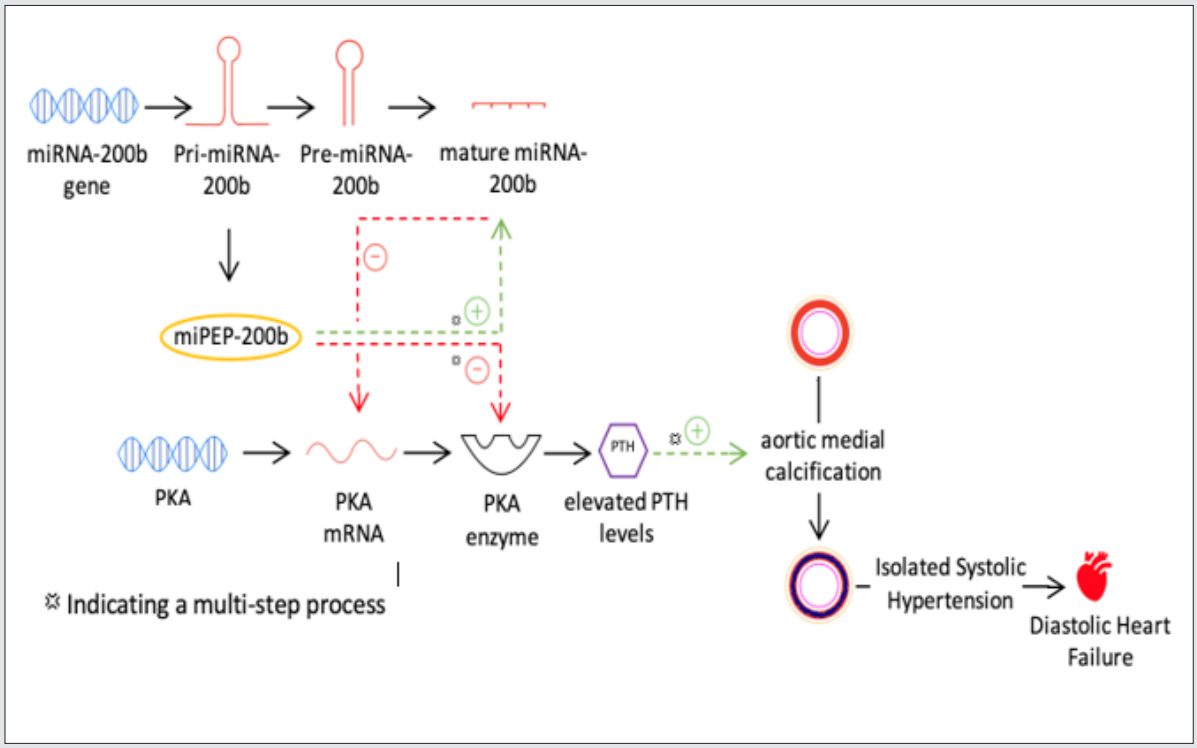

Figure 2: Proposed mechanism of aortic medial calcification pathogenesis (dashed red line: causing decreased activity of the target, dashed green line: causing increased activity of target).

Recently we have studied the expression of miPEP-200b in mammalian cells by raising polyclonal antibodies to miPEP-200b. Our results demonstrate that miPEP-200b is expressed in both breast and prostate cells (Figure 1). These results establish the presence of pri-miRNA-encoded proteins in mammalian cells. Many studies have been conducted on the implications of the miR-200 family in cancer development and repression; however, its association with cardiovascular pathology has not been a central area of focus. Because the miR-200 family has been shown to affect specific cellular pathways in various conditions, it would not be far-fetched to speculate on its implications in cardiovascular diseases known to have abnormalities in the same cellular pathways. It was found that a single gene variant for the miR-200 family could cause increased protein kinase A (PKA) activity, with subsequent thrombocyte activation and ensuing atherosclerosis [16]. In addition, PKA has been shown to act as a promoter of human smooth muscle cell (HSMC) calcification [17]. These processes are known to be the initial steps to the eventual development of vascular calcification [18]. Previously, we have shown that pri-miRNAs of 200a and 200b code for miPEP-200a and miPEP-200b respectively and these miPEPs function like miR-200a and miR-200b suggesting that Nature preserved this duplication of functions in case of a failure in any one of their functions. As such, we hypothesize that in the same way that miR-200b plays a role in decreasing PKA activity in atherosclerotic processes, the peptide miPEP-200b (encoded by pri-miR-200b) may also act as an inhibitor of PKA-induced HSMC calcification (Figure 2). Furthermore, targeted miPEP-200b, miRNA-200b, and PKA inhibitor therapy may lead to a substantial decrease in ISH development in older patients, with an ensuing decrease in the incidence and prevalence of diastolic heart failure secondary to longstanding hypertension. In this article, we will discuss the various components that provide evidence for these potential relationships and their sequelae.

Mirna and Mipep Interaction

Although there is a significant amount of information to be discovered regarding the functions of miPEPs, certain recent important findings allow us to predict potential activities of miPEPs in relation to miRNA. Of note is the study by Lauressergues et al. which showed that in plant cells, miPEPs behave as positive feedback on miRNA production [19]. It is possible that this relationship will also be shown in human physiology under controlled experiments in the future. This relationship is quite interesting, given the scarcity of positive feedback mechanisms observed in nature. In addition, various experiments in previous years involving miRNAs that attributed their observed findings to the effects of miRNAs alone may need to be revisited. As an example, it has been demonstrated that miRNA-200a and miRNA-200b are involved in the suppression of the epithelial-to-mesenchymal transition (ETM) in certain cancer types [20]. Although miRNA itself was originally thought to be the mediator of this observation, our recent findings demonstrate that the observed ETM suppression may be a result of miRNA alone, miPEP alone or a combination of both miRNA and miPEP activity [12]. Allowing this scenario to serve as a framework (Figure 3) in many other cellular pathways, one can imagine the vast number of cellular processes that may, in fact, have a different mechanism than what was previously proposed [21,22].

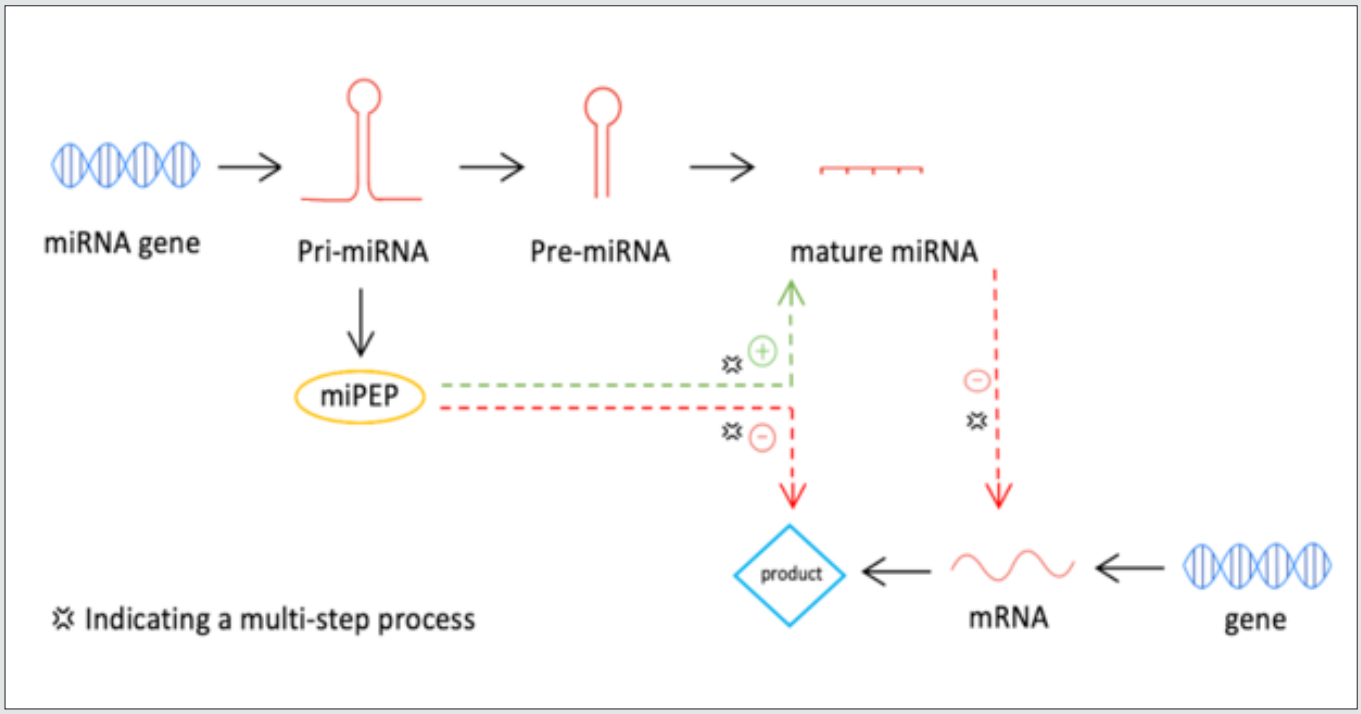

Figure 3: Proposed miRNA and miPEP relationship (dashed red line: causing decreased activity of the target, dashed green line: causing increased activity of target).

PKA and Aortic Calcification

The vasculature anatomy consists of 3 main layers: tunica intima, tunica media, and tunica adventitia. In the setting of aortic wall calcification, the media layer is involved. The main component of this layer is HSMC, which aids in constriction and dilation of vessels by contracting and relaxing, respectively (Figure 4). However, during the pathological process of medial aortic calcification, these cells begin to behave as osteoblasts, evident by upregulation of several markers associated with bone synthesis, including greater alkaline phosphatase activity [23]. Although there may be several mechanisms involved in this process, one explanation is the over-activity of PKA. PKA is an enzyme found in a multitude of cell types and tissues in the body. The enzyme is activated via cyclic AMP, and following activation, it is able to phosphorylate its substrates [24]. Abnormal PKA activity, therefore, causes various downstream effects. In particular, increased PKA activity may have important pathological implications in vascular calcification. There seems to be a potential mechanism involving PKA-induced elevation of parathyroid hormone (PTH). This may then induce medial aortic calcification [25]. with one experiment involving an in vivo model of rats with elevated PTH levels causing substantial calcification of the aorta [26]. In another study, it was shown that inorganic phosphate (Pi), which acts as a stimulator of PKA, led to HMSC calcification. In this experiment, HSMC were treated with Pi, calcium levels were measured, and subsequently, PKA inhibitors were added, then calcium levels were remeasured. It was demonstrated that inhibition of PKA activity by utilizing siRNA led to a greater than 50% decrease in HSMC calcium levels [27]. Although this study utilized siRNA as the inhibitor of PKA, Magenta et al. suggest that an increased PKA activity may be observed due to a single nucleotide polymorphism (SNP) where a thymidine nucleotide was substituted by cytosine in genes coding for the miR-200family [16]. This may suggest that the miR-200family plays a significant role in moderating PKA activity.

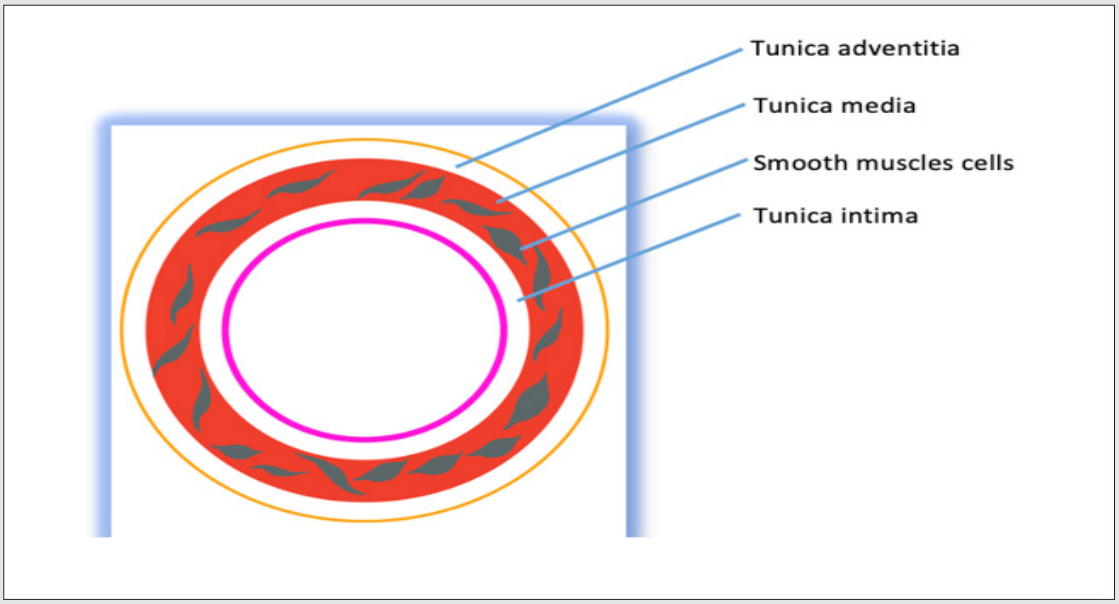

Figure 4: Various layers of arterial vasculature.

Aortic Calcification, Hypertension, and Heart Failure

A major sequela of vascular, specifically arterial, calcification is the development of hypertensive disease. As a result of the calcific process involving the media, the arterial system (including the aorta) becomes stiffened and loses its ability to dilate and decrease systemic vascular resistance. The resulting hemodynamic state is such that an isolated elevation in systolic blood pressure is observed, leading to both ISH and concomitant increased pulse pressure (difference between systolic and diastolic blood pressure). Although ISH has a strong association with medial calcification, its relationship with intimal atherosclerosis has not been elucidated to a significant degree [28]. With sustained, chronic hypertension, cardiac ventricular muscle cells undergo hypertrophy as a means to compensate for this increased afterload. Although this mechanism is compensatory, it is not without pathological consequence, with severe left ventricular hypertrophy (LVH) eventually leading to diastolic heart failure [29].

Discussion

Among the numerous functionalities that miRNA-200b has been shown to have [30], its suggested role in downregulating PKA activity may be an important player in preventing medial aortic calcification. This can be inferred as increased PKA activity has been associated with the deposition of bone-like material in the aortic medial layer. Although many cellular pathways may potentially be involved, one proposed mechanism is the PKA-induced increase in PTH levels. Interestingly, PTH is generally thought to be involved in bone resorption. However, it appears to have the opposite effect

leading to calcification in the vasculature. Following calcification of the aorta and other arteries, the stiffening of these vessels leads to ISH. With longstanding ISH, the myocardium becomes hypertrophied as a compensatory mechanism with the eventual development of diastolic heart failure. This proposed sequence is presented in Figure 1. Furthermore, miPEP may prove to be paramount in maintaining high miRNA activity, given the positive feedback that miPEP exerts on miRNA; although this observation has only been made in plant cells, the existence of both miPEP and miRNA in humans could provide the same relationship. No such studies have been published on the potential regulatory role of miPEP on miRNA in human physiology due to the relatively recent discovery of miPEP in our previous study [12]. A very recent study showed that miRNA-8 and miPEP-8 act to produce the same end result, regardless of their exact mechanisms of action, in *Drosophila* [31]. These findings are quite interesting, simulating many other protective mechanisms seen in nature, such as genetic redundancy [32]. By providing two mechanisms of accomplishing the same end goal, one mechanism serves as the main actor, and the other serves as the back-up in case of an event that renders one of them non-functional. As such, even in the case of a knock-out polymorphism of miRNA-200b, miPEP-200b can independently regulate the function of PKA by interfering with its regulatory subunit [16] and decreasing vascular calcification.

Conclusion

If our proposed mechanism of disease progression in medial aortic calcification in the context of miRNA-200b and miPEP-200b is shown to hold true, it will provide targets for therapeutic interventions. PKA antagonists, miRNA-200b, and miPEP-200b could be utilized with the end goal of decreasing PKA activity and decreasing vascular calcification with a subsequent decrease in the incidence and prevalence of both ISH as well as diastolic heart failure. Furthermore, there is potential for miRNA-200b and miPEP-200b levels to serve as prognostic factors for these diseases. There is reason to suggest this, as multiple studies have shown the promising potential of using miR-200 family levels as a strong prognostic marker for disease severity; low levels of the miR200 family have shown to be accurate predictors of a worse prognosis in pancreatic, lung, gastric, and bladder [33-36]. Although there is much to be discovered in this new arena involving miPEP, these findings provide an excellent starting point for future experimental designs, arming scientists with a new outlook on cellular pathways that were previously thought to behave differently.

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