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Editorial

Chemerin processing in the myocardium: A mechanism in search of a function

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Chemerin – also known as tazarotene-induced gene 2 protein or retinoid acid receptor responder protein 2 – is a signaling molecule that is widely expressed in most organs, including the heart [1,2]. It was first reported to promote chemotaxis of immune cells [1], a finding coherent with its initial discovery as a retinoid-responsive gene associated with the pathogenesis of psoriasis [3]: indeed, this papulosquamous skin disease is a pathology linked to autoimmunity and chronic inflammation. Also in line with its role as a chemoattractant, chemerin has been found to be significantly elevated in the blood of patients with other pathologies caused by an aberrant inflammatory reaction, such as pancreatic fibrosis [4], chronic hepatitis C [5], Crohn's disease, and ulcerative colitis [6]; in addition, it has been reported to be highly expressed in the synovial tissue of patients with rheumatoid arthritis [7]. To complicate matters, chemerin has been associated with improved survival in chronic renal disease [8], and it may have an anti-inflammatory action under particular circumstances [9–11], the mechanism of which may involve nonleukocytes [12].

More recently, chemerin has become implicated in the pathophysiology of adipose tissue. Apart from playing its classical role as a chemoattractant – in obesity being responsible for chronic inflammation of visceral fat [13], a histopathological phenomenon instrumental in the development of insulin resistance and other obesity-related comorbidities – chemerin may regulate adipogenesis and metabolism [2,14]. In actual fact, white adipose tissue may be a major source of the chemerin found in the circulation, and as such the protein is now classified among the adipokines – cytokines or hormones secreted primarily by adipocytes, but that may be produced to a lesser extent by other cell types too. It is thus a multifunctional molecule with primarily chemotactic and adipokine properties linked to the pathogenesis of diverse and widespread diseases.

The processing of chemerin is intricate. It is expressed as a pre-pro-protein by various cell types in addition to adipocytes, such as epithelia, endothelia, and fibroblasts, and is secreted as a poorly active ~18 kDa pro-protein that must be C-terminally cleaved – either in single or multi-step reactions – to produce a ~16 KDa, fully bioactive molecule [1]. A number of extracellular proteases, such as elastase, tryptase, and cathepsins, have been discovered to process prochemerin. Importantly, the specific enzyme catalyzing its proteolytic cleavage as well as the cell type secreting it determine the extent of C-terminal processing, resulting in the formation of different chemerin isoforms, each with differing bioactivity [15,16]. Once activated, chemerin binds with high affinity to its cognate receptor: G protein-coupled receptor chemokine-like receptor (CMKLR)1 [1,17,18]. CMKLR1 is highly present on immune cells and is abundantly expressed in lymph nodes, spleen, and lung; it is less expressed in other organs, such as the heart [1,2]. Signaling through CMKLR1 can take place *via* ERK1/2 [1,2,19–21], AKT [21], p38 [21], and NF-κB [22] pathways; in addition, the PKC pathway may be involved in internalization of the receptor–ligand complex, blunting the strength of the downstream signaling cascades [23]. Two other receptors can also bind chemerin: G protein-coupled receptor 1 (GPR1) produces only a weak effect, probably involving calcium mobilization [24]; and chemokine (CC motif) receptor-like (CCRL)2, which – in contrast to the other two receptors – does not seem to initiate a downstream signaling cascade, does not undergo ligand-induced internalization, and may function to present concentrated chemerin to CMKLR1 [25]. Any chemerin left extracellularly in the blood or extracellular matrix (ECM) is then degraded by further proteases, such as neutrophil proteinase, mast cell chymase, and angiotensin-converting enzyme [26]. This complexity in chemerin processing and signaling may allow for an orchestration of effects based on local tissue responses. Moreover, it highlights the need to study chemerin in individual cell types and tissue settings.

On this last point, Engelhardt and colleagues describe in a recent issue of the *Journal of Molecular and Cellular Cardiology* a novel mechanism that improves our understanding of chemerin processing and regulation in the myocardium [27]. In brief, the authors show that protease inhibitor 16 (PI16), a secreted protein that is highly upregulated in diseased myocardium, serves as an inhibitor of chemerin processing. The mechanism is thought to prevent untoward activation of local chemerin-dependent responses.

Going into more detail, Engelhardt and coll. find *Pi16* mRNA to be significantly more expressed by fibroblasts than by the myocardium's other cell types (cardiomyocytes, endothelial cells, and immune cells). Moreover, PI16 protein is bound to cell membranes *via* a glycophosphatidylinositol anchor at its C-terminal end. Of potential importance, secretion of the peptidase inhibitor into the cardiac ECM is significantly increased upon surgical induction of pressure overload *via* the transverse aortic constriction (TAC) procedure. However, their mouse with constitutional, general knockout of the *Pi16* gene did not present with an evidently different phenotype from wild-type control after being subjected to TAC. In fact, both strains developed similar levels of cardiac remodeling and functional impairment, at least up to 28 days from surgery, set as the experimentation endpoint.

Abbreviations: CCRL2, chemokine receptor-like 2; CMKLR1, G protein-coupled receptor chemokine-like receptor 1; ECM, extracellular matrix; GPR1, G protein-coupled receptor 1; PI16, peptidase inhibitor 16; TAC, transverse aortic constriction.

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The lack of a phenotype in *Pi16*-deficient mice at baseline and upon the induction of heart failure seems rather surprising in light of the authors' other findings. Indeed, the increased presence of PI16 in the myocardium of pressure-overloaded wild-type mice suggests that there is a link between the secretion of the inhibitor and some aspect of the pathogenesis of heart failure. In addition, Engelhardt had previously identified PI16 as an endogenous, paracrine repressor of cardiac hypertrophy, functioning in a feedback mechanism to control excessive growth-promoting stimuli [28]. In fact, *in vitro* RNAi-mediated suppression of *Pi16* increased basal cardiomyocyte size, whereas overexpression suppressed cardiomyocyte hypertrophy induced by exposure to adrenoreceptor agonists. *In vivo*, overexpression of PI16 in a cardiomyocyte-specific manner resulted in the formation of a small, but normally functioning, heart.

Engelhardt and coll. [27] hypothesize that the absence of an overt phenotype in $Pi16^{-/-}$ mice is due to the characteristics of the cardiovascular disease model employed (*i.e.*, TAC), in which cardiac remodeling is prevalent over inflammation, and/or to compensatory gene expression subsequent to constitutional deletion of the *Pi16* gene in the intact animal.

The authors then set out to ascertain a mechanism of action for secreted PI16. They noticed that a recent genome-wide association study had reported *rs1405069* – a single-nucleotide polymorphism located on the *Pi16* gene – to be among the variations associated most highly with increased circulating chemerin [20]. This was indicative of a link between PI16 and the adipokine. Indeed, Engelhardt and coll. [27] show that chemerin mRNA is expressed in the myocardium with a pattern reminiscent of that of PI16 – *i.e.*, significantly more present in cardiac fibroblasts than in other myocardial cell types; that the proteins encoded by the two mRNAs are functionally associated; and, finally, that PI16 inhibits the activity of the chemerin-activating protein cathepsin K. In fact, $Pi16^{-/-}$ myocardium has a high level of active chemerin.

Although downstream pathways in the cardiac setting are not specifically explored in their study, Engelhardt and coll. [27] hypothesize that chemerin released into the cardiac ECM activates CMKLR1 on responsive immune cells, leading to increased chemotaxis, recruitment, and pro-inflammatory activation of leukocytes. Certainly, there is some evidence of a role for inflammatory mediators in the pathogenesis of heart failure – a notion known as the cytokine hypothesis (for a review, read [29]). And the adipokine has already been linked to the pathogenesis of another cardiovascular disease with a strong inflammatory component: atherosclerosis. Indeed, local expression of chemerin by epicardial and aortic adipose tissue may be involved in the recruitment of macrophages to aortic and coronary atherosclerotic lesions [30]. However, quantitative data on leukocyte infiltration is not given by Engelhardt and coll. [27], so it is not known if there is any alteration in immune cell migration into the myocardium, a phenomenon reported to peak one week after TAC and become reduced thereafter [31,32].

In the cardiovascular setting, chemerin has been found also to have functions other than chemotaxis (Fig. 1). For example, it may induce cardiomyocyte death [33]. Indeed, CMKLR1 is expressed on the rat and mouse cardiomyocyte, and when these cells were cultured in the presence of chemerin, dephosphorylation of Akt at Thr308 and activation of caspase 9 lead to increased apoptosis. Thus, upregulation of PI16 secretion might have a potential anti-apoptotic effect. *In vivo* studies conducted on models of cardiovascular diseases with a prominent apoptotic component, such as myocardial infarction, may help to clarify this point.

Chemerin has been implicated also in angiogenesis [20,21]: *in vitro*, it induced formation of capillary-like structures by endothelial cells and so was hypothesized to support the growth of adipose tissue by stimulating vascularization. Whether this mechanism is operant in the vessels of the myocardium is not known.

Remaining in the sphere of vascular biology, chemerin has been more recently implicated as an endogenous vasoconstrictor [34]: in the presence of dysfunctional endothelium, secretion of the adipokine by perivascular adipose tissue resulted in contraction of vascular smooth muscle cells, another cell type expressing CMKLR1. Thus, there may be a link between chemerin and the development of hypertension. On this point, increased circulating chemerin has been positively associated with increased systolic and diastolic blood pressure [35–41].

Furthermore, chemerin may regulate myogenesis [42]: loss of CMKLR1 expression on skeletal muscle cells impaired their development *in vitro* – similarly to the effect observed by the same researchers in preadipocytes; *in vivo*, receptor knockout produced a general delay in embryonic development and a smaller body size. This latter finding is somewhat analogous to the observation of hypotrophic cardiomyocytes in the heart of *Pi16* transgenic mice [28], in which the activation of chemerin is presumably hampered. Signaling *via* CMKLR1 may also negatively modulate insulin-mediated glucose uptake into skeletal muscle, linking chemerin to myocyte metabolism.

Finally, epicardial fat thickness has been associated with cardiac dysfunction and disease [43,44]. Because the human heart lacks a fascia between this fatty tissue and the myocardium [45], adipokines secreted by it could directly act upon cardiac cells [46]. Whether chemerin is implicated in this route of adipose–myocardial signaling deserves investigation.

Over the last few decades, protein mediators of inflammation have gained much ground as important players in the pathology of the cardiovascular system. Regarding chemerin specifically, its level in the circulation has been variably associated with obesity and metabolic syndrome [35,47] – two pathologies intimately linked to the development of cardiovascular diseases – and with the progression of heart failure in patients with dilated cardiomyopathy [48]; moreover, chemerin has been proposed to a predictive marker of cardiovascular risk, severity of coronary artery disease [38,49–52], and progression of atherosclerotic lesions [53]. Its intricate processing and multifaceted pathophysiological role make it highly interesting but tough to study. Answering questions such as what specific signal upregulates IP16 expression in the presence of pressure overload, what myocardial cell type(s) is/are targeted by chemerin, and what isoforms are responsible for which effects, will undoubtedly further our knowledge of the mechanisms governing the development of heart failure. Studying the role of the PI16–chemerin pathway in other types of cardiovascular disease, such as myocardial infarction, may very well prove to be insightful too.

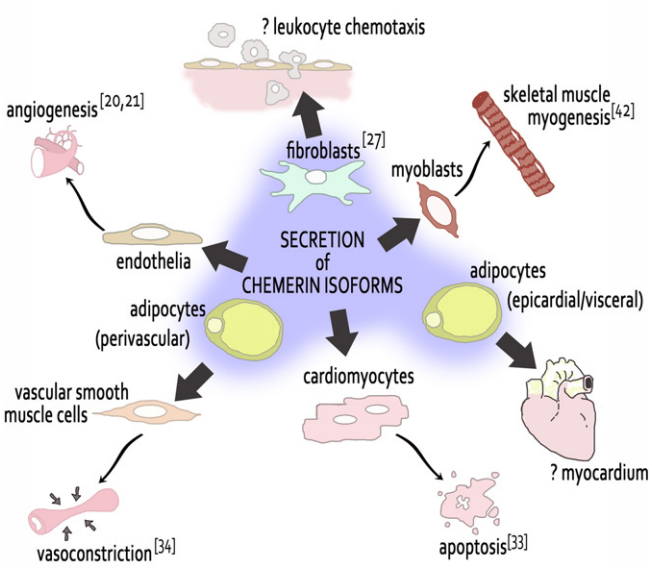

Fig. 1. Chemerin has been reported to play a number of pathophysiological roles in the cardiovascular system. Among these are chemotaxis, angiogenesis, vasoconstriction, myogenesis, and apoptosis (see text for explanations).