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Review

Proline-arginine rich (PR-39) cathelicidin: Structure, expression and functional implication in intestinal health

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ABSTRACT

Proline-Arginine-39 (PR-39) is a small cationic, proline and arginine rich, cathelicidin that plays an important role in the porcine innate immune system. Although PR-39 was first discovered in intestinal cell lysates of pigs, subsequent research has indicated that it is primarily expressed in bone marrow and other lymphoid tissues including the thymus and spleen, as well as in leukocytes. Mature PR-39 cathelicidin has anti-microbial activity against many gram-negative and some gram-positive bacteria. PR-39 is also a bridge between the innate and adaptive immune system with recognized immunomodulatory, wound healing, anti-apoptotic, and pro-angiogenic functions. The purpose of this review is to summarize our current knowledge about the structure, expression, and functions of PR-39 and its potential to promote intestinal homeostasis. This understanding is relevant in the search of alternative therapeutics against diarrheic enterocolitis, a major problem faced by pork producers both in terms of costs and risk of zoonosis.

Contents

1. Overview of PR-39

Cathelicidins are a major subgroup of mammalian host defense peptides that display both potent antimicrobial and immunoregulatory activities [1]. While human beings have only one cathelicidin

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(i.e. the 37-amino acid cathelin-associated peptide with amino terminal sequence, Leu-Leu; LL-37), other animals express a wide range of cathelicidin peptides. Swine (*Sus scrofa*) is a singular livestock species that expresses many linear α-helical cathelicidins such as PMAP-37 [2] and other cathelicidins with high secondary β-sheet content like protegrin-1 [1]. Among known porcine cathelicidins, the main cathelicidin identified to date is a PR-AMP with 39 amino acid residues in mature sequence, named PR-39 [3]. PR-39 peptide was first isolated from the small intestine of a pig exhibiting antibacterial activity against both *Escherichia coli* and *Bacillus megaterium* [4]. Four years after its discovery i.e. in 1995, the PR-39 gene was sequenced [5]. Recent research has indicated that PR-39 is a multi-functional peptide and a mainstay of the innate immune system. There is increasing evidence that PR-39 promotes angiogenesis, wound healing, leukocyte chemotaxis [6] and inhibits bacterial DNA and protein synthesis [3]. Although our understanding about AMPs has increased over past few years yet the question about diversity of the peptides across species still goes unanswered. Among many hypothesis, it is believed that the differential selective pressure, owing to variable microbial flora, diverse enteric and species specific environmental pathogens, is responsible for variable number of cathelicidins across different species [7]. Furthermore, functional differences could explain variable number of cathelicidins across species, for example, proline-arginine rich antimicrobial (PR-AMPs) PR-39 peptide chemoattracts leuckocytes [8], however no such function has been reported for PMAP. On the other hand, PMAP binds DNA more efficiently and therefore, is a better activator of dendritic cells than PR39, perhaps owing to the difference in the charge and structural properties of the peptides [9].

1.1. PR-39 gene sequence

The PR-39 gene is located on the telomeric porcine chromosome 13 at position 2.1 (13q2.1) and is comprised of 2967 base pairs [5]. Like other cathelicidins, the transcribed sequence of PR-39, rich in guanine and cytosine bases, is composed of four exons and three introns. The high degree of conservation of the intron sequences among distinct cathelicidins has captured the curiosity of researchers, and recent studies have confirmed that these introns have a functional role in upregulating transcription [10]. For instance, the second intron of the human cathelicidin LL-37, homologous to PR-39, was shown to elevate LL-37 expression levels in luciferase assays [11]. Unlike LL-37, upregulation of PR-39 is defined by TATA and CAT promoter sequences identified on the PR-39 gene, 24 and 130 base pairs upstream of the transcriptional start site, respectively [10,12]. The PR-39 gene sequence also contains six repeats of TGTCCTC, five repetitions in the first intron and one in the first exon, but the significance of this sequence repeats has not yet been determined [5,13].

1.2. PR-39 amino acid sequence and structural configuration

The PR-39 peptide is composed of 49% proline, 24% arginine, 13% phenylalanine, 5% lysine, 3% tyrosine, 3% isoleucine, and 3% glycine [5]. Similar to other cathelicidins, PR-39 is synthesized as a precursor called a prepropeptide of 173 amino acids [5], and it is only after multiple cleavage steps that the mature 4719 Da PR-39 protein is formed [14] (Fig. 1). While not contained in the mature peptide, the cathelin domain makes up the largest part of the PR-39 prepropeptide. The cathelin domain refers to the highly conserved residue sequence coded primarily by the first three exons of the processed mRNA strand of the PR-39 gene, and is a domain shared among all mammalian cathelicidins [15]. The first exon of the processed mRNA strand codes for a short untranslated region, a 29-residue signal sequence and the first 37 residues of the cathelin domain. Together, the second and third exons encode the next 60 amino acids of the cathelin pro-peptide. The last exon is translated to yield the final four residues of the cathelin domain, the 39 amino acids of the mature PR-39 peptide, as well as a terminal GKR residue sequence [5,10,16] (Fig. 2).

The amino acid sequence of PR-39 results in a peptide that is amphipathic, linear, and positively charged (+10) [3]. The primary structure of PR-39, rich in proline, inhibits the formation of alpha helices and beta sheets, but extensive evidence shows that the peptide contains type II poly-L-proline helix conformation (phi = −78 psi = 149 and three residues per turn) similar to collagen [14,17]. In addition, the mature PR-39 peptide possesses SH3 residue binding motifs that are often found in type II poly-L-proline helix structures [14,18]. The polyproline structure of PR39 is essential in the blocking of bacterial DNA and protein synthesis as its amphipathic structure facilitates penetration on bacterial plasma membranes and causes lipid destabilization [18]. The high proline content of processed PR-39 also prevents its degradation by serine proteases while its elevated arginine content leaves it with a positive net charge. The positive charge on the peptide contributes to the antimicrobial activity through promotion of electrostatic interaction with negatively charged bacterial membranes [3]. Extensive research has been performed on the first 15 amino acids of PR-39, as this region is highly similar among all porcine cathelicidins. These conserved motifs include a N-terminal arginine and the RPRP sequence at position ten [18]. The N-terminal arginine is key in the functioning of SH3 binding domains, peptide function, and antimicrobial activity, while the RPRP sequence is needed for the stabilization of the type II polyproline helix [18]. In addition, the stereochemistry of PR-39 is important for function as all-D-amino-acid analogs have inferior microbicidal activity than L-amino-acid peptides [6].

2. Expression of PR-39 in the porcine intestinal tract

While PR-39 was originally isolated from upper part of small intestine, subsequent research has indicated that the primary source of intestinal PR-39 is more likely to be neutrophil granules [6] or resident leukocytes [13] that secrete cathelicidins into the intestinal intercellular space. The secretion of PR-39 in the intestine from bone marrow derived cells has been demonstrated in pigs orally challenged with *E. coli*; PR-39 mRNA levels were highest in the bone marrow followed by spleen, mesenteric lymphoid node, thymus, liver, and ileal tissues [19]. Likewise, pigs challenged with *Salmonella enterica* Typhimurium (S. Typhi) showed increased levels of PR-39 mRNA in bone marrow derived cells at 6 h and 24 h post-challenge [20]. The PR-39 response in bone marrow to enteric bacterial pathogens seems mostly induced by membrane components of gram-negative bacteria (i.e. lipopolysaccharide; LPS), which has shown to increase the mRNA expression of PR-39 in mononuclear and granulocytic-lineage cells [20]. Thus, the amount of PR-39 in intestinal tissue is likely related to the abundance of myeloid cells within the tissue [19]. Infiltration of polymorphonuclear neutrophils (PMNs) and macrophages with ensuing release of cathelicidin in responses to bacteria infection in pigs has been well characterized in other organs, including the respiratory tract. In a study that examined pigs infected with *Actinobacillus pleuropneumoniae,* the presence of the bacteria in PMNs and macrophages was associated with the detection of PR-39 in the upper airway, and lung and lympathic tissues [21]. PR-39 has been also found in increased quantities in wound fluid isolated from cultured mesenchymal cells [22]. This could be because of augmented expression of PR-39 in the intestine that can occur as a reparative response at the site of injury, as cathelicidins can promote wound healing and immune response following injury [22].