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Mini review

Defensins: "Simple" antimicrobial peptides or broad-spectrum molecules?

Meggy Suarez-Carmona [a,b], Pascale Hubert [a], Philippe Delvenne [a], Michael Herfs [a,*]

[a] Laboratory of Experimental Pathology, GIGA-Cancer, University of Liege, CHU-Sart Tilman, 4000 Liege, Belgium
[b] Laboratory of Tumor and Development Biology, GIGA-Cancer, University of Liege, CHU-Sart Tilman, 4000 Liege, Belgium

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ABSTRACT

Small cationic peptides highly conserved in vertebrates, both α- and β-defensins were primarily identified as anti-microbial compounds involved in innate immunity. While human α-defensins are mostly expressed by neutrophils, β-defensins are secreted by epithelial cells of the skin and mucosae. Besides their anti-microbial activity, accumulating data emerged in the past decade indicating that defensins have extended functions in human physio(patho)logy. Indeed, defensins appeared as modulators of the adaptive immune system and angiogenesis, key mediators of wound healing and determinant players in male fertility. Furthermore, the impact of defensin expression in cancer and the potential use of these small peptides as biomarkers or even therapeutic tools should not be ignored. In the present review, we describe recent research works regarding the diversified functions of defensins, by mainly focusing on human models.

1. Introduction

Members of the antimicrobial peptide superfamily, defensins are small (~4–6 kDa) cationic peptides secreted in various species including humans and other mammals but also fishes, birds, insects, filamentous fungi, plants, etc. This wide distribution suggests that the production of such peptides is an ancient and well-conserved mechanism of host defense [1]. In humans, defensins are classified into two subgroups (α and β), based on both unique amino acid sequences and disulfide connectivities (Table 1). Primarily detected in neutrophils, α-defensins 1–4, known also as human neutrophil peptides (HNP), are also expressed in NK cells, monocytes and some T lymphocytes subsets. Human α-defensins (HD) 5 and 6 were initially detected in Paneth cells of the small intestine suggesting a role of protecting the intestinal stem cells within the crypt [2]. Moreover, the expression of these peptides was also reported in both respiratory and *gynecological tracts* [3–5]. Although genomic analyses reported at least 26 transcriptionally active human β-defensin (HβD) genes [6], to date, only a few have been cloned, isolated and fully characterized at the protein level. Whereas HβD1-4 are secreted by a large variety of mucosal epithelia including those lining urogenital, gastrointestinal and respiratory tracts [7], HβD5-6 genes seem to be specifically expressed in the epididymis [8]. Other human epididymal β-defensins (HβD14, HβD18, HβD23 and HβD26 encoded by DEFB114, DEFB118, DEFB123 and DEFB126, respectively) were also identified [9–11]. These latter peptides are, however, still largely unknown.

Originally discovered due to their antimicrobial activity, accumulating data suggest that the collective functions of defensins extend well beyond their activities in innate immunity. The present review is mainly based on recent studies on both α- and β-defensins of human origin and highlights the recent advances in the involvement of these arginine-rich peptides in the immune system, injury and carcinogenesis. The potential diagnostic/clinical relevance of their broad effects is also addressed.

2. Defensins: synthesis, processing, conformation and regulation

Despite the lack of similarity at the genetic level, all human defensins are synthesized *in vivo* as large prepro-peptide (up to ~110 amino acid residues) consisting of an N-terminal extension followed by the pro-peptide. This latter is composed of the pro-domain and the C-terminal mature defensin sequence (~35 residues) [12]. Typically very short and devoid of any inhibitory function in HβD pro-peptides, the pro-domain in α-defensin precursors contains ~40 amino acids and is essential to maintain the biologically active peptide in an inactive state. Through the

* Corresponding author. Tel.: +32 43664282; fax: +32 43662919.
E-mail address: M.Herfs@ulg.ac.be (M. Herfs).

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Table 1
Expression patterns of main human alpha- and beta-defensins.

	Defensin	Gene (location)	Main cell/tissue sources	Synthesis and stimuli
α-Defensins	HNP 1	DEFA1 (8p23.1)	Neutrophils, monocytes, macrophages, NK cells	Constitutive
	HNP 2	DEFA2 (8p23.1)		
	HNP 3	DEFA3 (8p23.1)		
	HNP 4	DEFA4 (8p23)		
	HD-5	DEFA5 (8p23.1)	Paneth cells, cervico-vaginal epithelial cells	Constitutive (but upregulated in the case of bacterial infection)
	HD-6	DEFA6 (8p23.1)		
β-Defensins	HβD 1	DEFB1 (8p23.1)	Epithelial cells (lining numerous organs)	Constitutive
	HβD 2	DEFB4A (8p23.1)	Epithelial cells (lining numerous organs)	Inducible by bacterial, fungal and viral products or proinflammatory cytokines
	HβD 3	DEFB103 (8p23.1)		
	HβD 4	DEFB104 (8p23.1)		
	HβD 5	DEFB105 (8p23.1)	Epididymis	Unknown
	HβD 6	DEFB106 (8p23.1)		
	HβD 14	DEFB114 (6p12.3)		
	HβD 18	DEFB118 (20q11.21)		
	HβD 23	DEFB123 (20q11.1)		
	HβD 26	DEFB126 (20p13)		

interaction with the mature peptide, the pro-segment acts as an efficient intramolecular inhibitor avoiding autocytotoxicity [13,14]. During neutrophil differentiation, HNP1-4 precursors would be excised by neutrophil elastase and proteinase 3 [15]. Resulting mature α-defensins are then stored in azurophilic granules which are released into phagocytic vacuoles containing ingested pathogens. Although neutrophils express various enzymes (*e.g.* elastase, proteases) and other bactericidal polypeptides (*e.g.* lactoferrin), HNPs constitute >40% of the total protein content within the azurophilic granules [16,17]. Almost undetectable in the extracellular fluids in normal healthy individuals, the plasma level of HNPs may reach concentrations up to 100 mg/ml in the setting of sepsis or bacterial meningitis [18]. This release of a large amount of HNPs is a consequence of neutrophil degranulation (exocytosis), lysis and/or leakage during phagosome formation [19]. In contrast to HNPs, processing of HD-5 in the intestinal crypt occurs extracellularly and is mediated by a trypsin isoform [20]. Interestingly, multiple processing intermediates of HD-5 and HD-6 were observed *in vivo*, especially in the female reproductive tract [3]. However, the proteases involved in the generation of these naturally occurring forms remain unknown. Regarding HβDs, a mixture of forms containing variable numbers of amino acids (between 36 and 47 residues in length) was detected in urine, blood plasma and cervicovaginal lavage, reflecting variable N-terminal proteolytic processing [21]. Despite the relatively minor changes in the peptide sequence, HβD intermediates display marked differences in their functional activity. Similar to HNPs, the enzyme(s) involved in HβD propeptide processing is still subject to investigations. Both HNP1 and HβD1 were recently identified as targets for matrix metalloproteinase 7. However, its apparent absence of expression in both squamous epithelia and neutrophils suggests that this protease is not involved in the intracellular processing of pro-defensins [22].

Both α and β defensins adopt a relatively compact molecular conformation with rigid β-sheet structures that are stabilized by three intramolecular disulfide bonds. However, as mentioned above, the disulfide-pairing pattern is specific for each defensin subgroup. Indeed, α-defensins are linked by cysteines 1–6, 2–4, and 3–5, whereas the six cysteine residues in HβDs are connected 1–5, 2–4 and 3–6 [7,23]. Although these disulfide connectivities are essential for the CCR2/CCR6-mediated chemotactic activities of defensins [24], unexpectedly, several studies demonstrated that they are not required for their antimicrobial activities. For example, linear unstructured HD-5 and HβD3 analogs exhibited similar anti-HIV and antimicrobial (against *Escherichia coli*) activities, respectively [24,25]. However, one exception was recently reported. In reducing environment, an increased antimicrobial activity of HβD1 against commensal bacteria and pathogens was demonstrated suggesting that redox-regulation could modulate the structure of HβD1 and, therefore, its function in mucosal surfaces [26].

Whereas HNPs are abundantly and constitutively expressed, in particular, in neutrophils, HD-5 and HD-6 basal expression levels are modulated in response to sexually transmitted infections (urethral secretions) [27] or to a down-regulation of the Wnt-signaling pathway (Tcf4 transcription factor in Crohn's disease) [28]. A recent study also identified IFN-γ as a trigger for HD-5 release in the intestinal epithelium [29]. Regarding HβDs, a ubiquitous expression of HβD1 was observed in all epithelia irrespective of their differentiation [2,7]. In mucosal surfaces lined by a squamous epithelium, ΔNp63 isoforms would be involved in the constitutive HβD1 mRNA expression, as recently demonstrated in squamous cell carcinoma [30]. However, this "master regulator" of squamous differentiation cannot explain the HβD1 secretion observed in both columnar and ciliated epithelia [5]. In contrast to HβD1, HβD2 and HβD3 are induced in inflamed/infected mucosa by bacterial, fungal and viral products or proinflammatory cytokines [*e.g.* interleukin (IL) 1β and tumor necrosis factor (TNF) α] [7,31]. These two latter soluble factors do not seem to affect HβD4 expression [32]. Although epidermal growth factor receptor (EGFR) signaling pathway could also participate in HβD up-regulation [33,34], the HβD synthesis is predominantly mediated by NF-κB and *Mitogen-activated protein kinase* (*MAPK*) pathways leading to AP-1 transcriptional activation [35]. Indeed, consensus NF-κB and AP-1 binding sites are present in the gene promoters of all inducible HβDs. Through the inhibition of AP-1 activity, retinoic acid has been shown to completely abolish HβD expression in keratinocytes. These results suggest that the balance between active metabolites of vitamin A and proinflammatory cytokines could be critical for HβD regulation in mucosal surfaces [36].

3. Defensins as antimicrobial peptides

All human defensins have demonstrated the ability to exert antimicrobial activities (killing and/or inactivation) over a wide variety of bacteria, viruses, fungi and protozoa. Although further investigations are still needed, the direct microbicidal mechanisms of these cationic effectors of innate immunity are presumably initiated by an interaction with the negatively charged membranes of pathogens [37,38]. The variability in the phospholipid composition of the bacterial membranes or viral envelopes might explain the differential antimicrobial effects of defensins which have been highlighted in numerous studies. Similarly, human lipid bilayer