Cathelicidins Revisited: Molecular Evolution, Structure and Functional Implications

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ABSTRACT

Cathelicidins constitute important antimicrobial peptides of innate immunity. In order to elucidate the evolutionary history of cathelicidins, the author performed comprehensive phylogenetic analyses of cathelicidin homologs in all available genomes including those completed recently. The organization of cathelicidin genes, as well as the secondary and tertiary structures of the inferred proteins are conserved. Based on integrated genomic, structural, and functional data, the author identified the last common ancestor of the cathelicidin family in lampreys, thus tracing the evolutionary origin of cathelicidins 550 million years ago. The author’s data suggest that cathelicidins arose concordantly with the adaptive immune system, a new organismal function first acquired in lampreys. The appearance of cathelicidins at the junction of innate and adaptive immunity may explain their dual roles as signal transducing molecules and as antimicrobial peptide precursors. Conserved regulatory elements associated with functions of the immune system were identified in cathelicidin gene promoter sequences invariably from fishes to humans.

Keywords: Adaptive Immunity, Cathelicidins, Cystatins, Evolution, Innate Immunity

INTRODUCTION

The innate immune system developed before the separation of vertebrates and invertebrates. In contrast to the acquired immune response, the innate immune system confers broad specificity, and provides immediate and effective defense against infection without previous exposure to a pathogen (Kimbrrell & Beutler, 2001). Nonetheless, in the absence of innate immunity, the adaptive immune response offers weak protection against infection (Kimbrrell & Beutler, 2001). Antimicrobial peptides (AMPs), including cathelicidins, constitute an integral part of innate immunity. More specifically, cathelicidins provide the first line of defense against a broad spectrum of invading pathogens including both Gram-positive and Gram-negative bacteria, fungi and viruses (Barbeiro et al., 2013; Veldhuizen, Brouwer, Schneider, & Fluit, 2013; Wantha et al., 2013; Zanetti, 2005). Cathelicidin peptides have been identified thus far in vertebrates, like mammals including humans (Gudmundsson et al., 1996) and primates (Zelezetsky et al., 2006), mouse (Gallo et al., 1997), guinea pig (Nagaoka,
Tsutsumi-Ishii, Yomogida, & Yamashita, 1997), bovine (Skerlavaj et al., 1996), pig (C. Zhao, Ganz, & Lehrer, 1995), dog (Sang et al., 2007), rabbit (Tossi, Scocchi, Skerlavaj, & Gennaro, 1994), horse (Scocchi et al., 1999), sheep and goat (Shamova et al., 1999), panda (Yan et al., 2012), tammar wallaby (Daly et al., 2008), birds including chicken (Goitsuka et al., 2007; Xiao et al., 2006), and pheasant (Wang et al., 2011), reptiles including snakes (Wang et al., 2008; H. Zhao et al., 2008), amphibia (Hao et al., 2012) and fish including cod and salmon (Chang, Zhang, Zou, Nie, & Secombes, 2006; Scocchi, Pallavicini, Salgaro, Bociek, & Gennaro, 2009). The evolutionarily basal (closest to the origin) cathelicidins were identified in the primitive Atlantic hagfish Myxine glutinosa (Uzzell, Stolzenberg, Shinnar, & Zasloff, 2003).

Cathelicidins exert direct microbicidal effects but also mediate various immune-modulatory functions including stimulation of components of the adaptive immune system, chemoattraction of neutrophils, angiogenesis and wound repair (Hancock & Sahl, 2006). Due to these pleiotropic functions, cathelicidins are also referred to as “alarmins” (Oppenheim & Yang, 2005).

The cathelicidin genes are expressed as inactive precursors mostly in the cytoplasmic granules of circulating neutrophils/polymorphonuclear leukocytes (PMN) and to a lesser extent in hematopoietic cells, epithelial cells or skin keratinocytes. Proteolytic processing of the cathelicidin precursor protein is a prerequisite for their antimicrobial activity. First, the signal peptide is removed by a signal peptidase to generate the proform, which includes the N-terminal cathelin-like domain (CLD) and the antimicrobial domain (AMD). The CLD is an evolutionarily conserved anionic domain of approximately 100 amino acid residues, while the cationic AMD is a hypervariable, both in size (12-100 residues) and sequence (Hancock & Sahl, 2006; Shinnar, Butler, & Park, 2003; Zanetti, 2005). The CLD belongs to the same family with cystatins, a superfamily of endogenous inhibitors of cysteine proteases (Zhu, 2008a). Although its function has not been elucidated, CLD is proposed to bind electrostatically to the cationic AMD in order to prevent its antimicrobial activity until release (Shinnar et al., 2003). The C-terminal AMD peptide can interact and destroy both Gram-positive and Gram-negative bacteria, protozoa, and fungi through electrostatic interactions with the negatively charged molecules found on the cell surface of their targets (Hancock & Sahl, 2006). The cathelicidin AMDs adopt all major structural conformations of antimicrobial peptides, including α-helical and amphipathic structure, linear tryptophan-rich, proline-rich, and β-hairpin structure. The full range of the above structural conformations has been identified in cetartiodactyla (Shinnar et al., 2003; Tomasinsig & Zanetti, 2005; Zaiou & Gallo, 2002; Zanetti, 2005).

Cathelicidin-encoding genes are approximately 2 kb in size with conserved four exon/three phase-0 intron (I\textsubscript{0}) organization. The signal peptide and the CLD are encoded by exon I to III, while the cleavage site, AMD and the 3′ UTR are encoded by exon IV (Betts, Guigo, Agarwal, & Russell, 2001; Zhu, 2008a). According to Zhu (2008a), cathelicidins have likely evolved from cystatins by acquiring a third intron (I\textsubscript{03}), in addition to the introns I\textsubscript{01} and I\textsubscript{02} of cystatins, via a reverse-transcription-mediated mechanism. Similar to cystatins, the characteristic structural features of CLDs include an α-helix cradled by a five-stranded β-sheet and conserved disulfide bridge patterns (Tomasinsig & Zanetti, 2005; Zaiou & Gallo, 2002). Consistent with the structural conservation between cystatins and CLDs it was demonstrated that the human CAP18 CLD was able to inhibit the activity of human cathepsin L protease (Zaiou, Nizet, & Gallo, 2003). However, the pig protegrin-3 (PG3) CLD was found to activate rather than inhibit cathepsin L. This activating effect is associated with the L2 loop which is important for the functional interaction of cystatin inhibitors with their target proteases (Zhu, 2008a). Interestingly, human CLD was found also to exert antimicrobial function (Zaiou et al., 2003).

The availability of an increasing number of complete or almost complete genomes allowed