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Rapid communication

Molecular identification and functional expression of porcine Toll-like receptor (TLR) 3 and TLR7

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Abstract

To investigate porcine Toll-like receptors (TLR) responding to viral pathogen associated molecular patterns, the full-length cDNA of porcine TLR3 and TLR7 were identified and characterized. Porcine TLR3 and TLR7 cDNA encode 904- and 1050-amnio-acid polypeptides, respectively. Both porcine TLR3 and TLR7 contain typical functional TLR domains and share about 80% sequence identity to other mammalian orthologues. Tissue expression profiles showed that TLR3 was highly expressed in kidney, duodenum, spleen and liver, and moderately expressed in bone marrow, lung, and skin. Conversely, TLR7 was moderately and constitutively expressed in all tissues evaluated. Stimulation of mammalian cells transfected with porcine TLR3 and TLR7 constructs with TLR3 and TLR7 agonists [poly (I:C) and imiquimod (R837), respectively], and adenovirus elicited activation of interferon regulatory factors (IRFs). These data provide molecular and functional information for porcine TLR3 and TLR7, and implicate their role in mediating immune protection against porcine viral diseases.

Keywords: Porcine; TLR3; TLR7

Toll-like receptors (TLRs) are pathogen recognition receptors that are primary components of the afferent arm of innate immunity (Pichlmair and Reis e Sousa, 2007; Takeuchi and Akira, 2007). Among the more than ten TLRs that have been identified in mammals, four receptors, TLR3, TLR7, TLR8 and TLR9, are involved notably in virus recognition. For example, TLR3 detects double-stranded RNA (dsRNA) formed during viral genome replication or transcription; TLR7 and TLR8 recognize elements of single-stranded RNA (ssRNA) found in genomes of RNA viruses; and TLR9 senses unmethylated cytosine–phosphate–guanine (CpG) motifs common to both bacterial and viral DNA (Barton, 2007; Pichlmair and Reis e Sousa, 2007; Takeuchi and Akira, 2007). Unlike TLRs located on the cell surface such as TLR1–TLR6 and TLR10–TLR13,

Abbreviations: CpG, cytosine-phosphate-guanine; dsRNA, double-stranded RNA; EGFP, enhanced green fluorescent protein; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IRF, interferon regulatory factor; poly (I:C), polyinosinic-polycytidilic acid; RACE, rapid amplification of cDNA ends; ssRNA, single-stranded RNA; TLR, Toll-like receptor.

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Table 1
Primers used for PCR and RT-PCR

Primers	Primer sequences (5′–3′)	GenBank™ accession numbers	Location in cDNA (nt)
TLR3 RACE		DQ647698	
5′ RACE-inner	TTGCCGGGAGGTCATCGGATATTT		555–531
5′ RACE-outer	AGCTGAGAGAGCTCATTGTGTTGG		741–718
3′ RACE-inner	GCACCATGCAGTTCAGCAAGCTAT		2893–2916
3′ RACE-outer	CAGGTGCCCTTGAACTTGAAGCAA		2781–2804
TLR3 RT-PCR			
Sense 1	AAGAACTCACAGGCCAGGARTGGA		1731–1754
Antisense 1	AGCCGTGCTAAGTTGTTATGCTGM		2032–2009
TLR3 clone			
Sense	gg*aagctt*ATGTCTACCTACTTATGTACTGTTAGACAT[1]		470–496
Antisense 1	ccggatcccgATGTACTGAATTTCTTGAACCAAG[1]		3132–3109
Antisense 2	ccggatccGCACTGTCTTTGCAGGGTGATGT[1]		2520–2498
TLR7 RACE		DQ647699	
5′ RACE-inner	AGTAACAGTTCTGGCCCAGGTAGA		672–649
5′ RACE-outer	AGGGCAATTTCCACTTAGGTCCAG		902–879
3′ RACE-inner	AAATCCACAGGCTCACCCGTACTT		3173–3196
3′ RACE-outer	TCACCCAATTCCTGCTACGATGCT		2778–2801
TLR7 RT-PCR			
Sense	ACAATGATATCGCCACCTCCACCA		1936–1959
Antisense	TGGCCAAGGAGAGAGTCTTCAGAT		2172–2149
TLR7 clone			
Sense	gg*aagcttATG*GCTAGATGGTTTCCTAAAACTCTG[1]		197–220
Antisense 1	gaccgcggTGTCTCTTTGAACACCTGACT[1]		3266–3246
Antisense 2	gaccgcggATGGTTAACCCACCAGACAAG[1]		2522–2502

[1] Underlined segment was introduced for cloning.

virus-sensing TLRs are located mainly in endosomes, which is where viruses undergo de-coating during infection (Pichlmair and Reis e Sousa, 2007). Structurally, all identified TLRs contain a ligand-binding, leucine-rich extracellular domain, a transmembrane region, and a conserved Toll/IL-1 receptor (TIR) domain, which transduces perceived signals and induces expression of immune responsive genes (Barton, 2007; Gay and Gangloff, 2007). Prominently in antiviral responses, TLR-mediated signaling pathways activate core transcription factors including nuclear factor (NF)-κB and interferon regulatory factors (IRFs), such as IRF-3 and IRF-7, which subsequently induce the production of type I IFN, a hallmark of antiviral immune responses (Barton, 2007; Kawai and Akira, 2006; Pichlmair and Reis e Sousa, 2007; Severa and Fitzgerald, 2007).

Although several porcine TLRs have been identified (Meier et al., 2004; Shimosato et al., 2005; Shinkai et al., 2006a,b; Tohno et al., 2005, 2006), identification and studies on porcine TLRs responding to viral pathogen associated molecular patterns are limited. Porcine TLR9 has been identified and shown to be expressed in intestinal Peyer's patches and expression was stimulated in monocytes and monocyte-derived dendritic cells after treatment with synthetic poly (I:C) and CpG oligonucleotides (Tohno et al., 2006). In addition, a complete cDNA for TLR8 has been identified and deposited in GenBank™, accession number NM 214187. Here, we report the identification and initial characterization of porcine TLR3 and TLR7. Both TLRs were expressed in immune tissues, show 76–90% identity to other mammalian orthologues, and conserve the typical TLR domains. Furthermore, gain-of-function experiments showed that both TLRs augment the activation of IRFs. Collectively, these findings provide a molecular foundation to examine the role of porcine TLR3 and TLR7 in mediating immune responses against porcine viral diseases.