

## 猪Toll样受体4基因SNPs功能分析

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**摘要** Toll样受体4(Toll-like receptor 4, TLR4)在机体的免疫反应中发挥重要作用, 该基因突变会影响受体的信号转导能力和机体的疾病抗性/易感性。文章在前期工作的基础上, 进一步分析c.611 T>A (p.Leu204His)、c.1027 C>A (p.Gln343Lys) 和c.1605 G>T (p.Leu535Phe) 3个错义突变对猪TLR4功能的影响。利用RT-PCR方法克隆猪TLR4基因全长编码区并引入定点突变; 利用真核表达、双荧光素酶报告系统和Western blotting方法在瞬时转染的PK-15细胞内研究3个单核苷酸多态(Single nucleotide polymorphisms, SNPs)对猪TLR4配体识别和信号转导能力的影响; 同时, 利用创造酶切位点PCR-RFLP方法分析对TLR4活性有显著影响的点突变在民猪、大白、长白和中国东北野猪4个群体中的分布。结果, 成功获得了民猪TLR4基因的全长编码区和3个单碱基变异体, 构建了不同等位基因的真核表达载体, 在PK-15细胞内确定了c.1605 G>T变异导致TLR4向下游传递信号的能力显著降低(P<0.01), 该SNP只存在于民猪和野猪中且频率较高。猪TLR4基因c.1605 G>T变异影响Toll样受体的信号传递, 可能和机体的疾病抗性/易感性有关。

**关键词:** 猪 TLR4 SNP 功能分析

**Abstract:** Toll-like receptor 4 (TLR4) plays an important role in immune response and the polymorphism in it might affect protein signaling and host resistance/susceptibility to disease. This study was designed to characterize the functional relevance of 3 nonsynonymous single nucleotide polymorphisms (SNPs), c.611 T>A (p.Leu204His), c.1027 C>A (p.Gln343Lys), and c.1605 G>T (p.Leu535Phe), which were selected based on our previous studies. RT-PCR method was used to clone the complete coding sequence of porcine TLR4 gene and the PCR-based method was used to introduce the point mutation. The effects of 3 SNPs on the ligand recognition and signaling of porcine TLR4 were investigated in transiently transfected PK-15 cells using dual-luciferase reporter system and Western blotting method. At the same time, the distribution of c.1605 G>T among pig populations composed of Min pig, Yorkshire, Landrace, and Wild boar from north-eastern China was studied by created restriction site PCR-RFLP method. The complete coding sequence of TLR4 gene in Min pig and 3 variants with single point mutations were obtained. Eukaryotic expression vectors containing different alleles of porcine TLR4 were constructed. SNP c.1605 G>T significantly decreased the TLR4 signaling (P<0.01) and the polymorphism only existed in Min pig and Wild boar from northeastern China with high frequencies. SNP c.1605 G>T in porcine TLR4 might affect the receptor function and host resistance/susceptibility to diseases.

**Keywords:** porcine, TLR4, SNP, functional analysis

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