

绵羊MHC区段3个预测基因的验证与表达分析

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摘要 对新疆美利奴细毛羊基因组MHC(Major histocompatibility complex)区段细菌人工染色体(BAC)文库的DNA序列进行测定, 经过序列比对分析, 首次预测了约130个新基因, 其中有8个CDS(Coding sequences)未在其他物种中发现其同源序列, 推测可能系绵羊所特有。在此基础上, 文章对绵羊MHC区段预测的3个新基因(分别命名为*OaN2*、*OaN5*、*OaN6*)进行了实验验证和表达分析。从绵羊肺组织中克隆到了*OaN2*的cDNA序列, 其长度为270 bp; 从肠系淋巴结中扩增得到 *OaN5*和*OaN6*的cDNA序列, 长度分别为309 bp和205 bp。上述3个基因的GenBank登录号分别为JF330782、JF330783和JF330784。利用Northern blotting技术进行转录本水平分析, 发现这3个新基因均在免疫器官肠系淋巴结中高表达。通过Western blotting和原位免疫组化技术对*OaN2*蛋白水平进行了表达谱分析, 结果表明*OaN2*蛋白在绵羊脾脏和肠系淋巴结等免疫器官中高表达, 在心、肝及胰脏中不表达。这是首次通过实验验证绵羊MHC区段的3个预测的新基因, 为其在绵羊免疫器官中的功能研究奠定了基础。

关键词: 绵羊 MHC 新基因 表达分析

Abstract: Previous DNA sequencing of BAC clones covering entire ovine MHC (OLA) region resulted in identification of approximately 130 functional genes in the region, of which 8 were predicted by computer software to be exclusively existed in sheep, but not in any other species known to date. In the present study, we successfully identified and cloned cDNA sequence of *OaN2*, *OaN5*, and *OaN6* from representative sheep tissues, confirmed their existence in reality. The sequences obtained experimentally exactly identical to those predicted previously. The length of cDNA fragments for *OaN2*, *OaN5*, and *OaN6* was 270 bp, 309 bp, and 205 bp, respectively, with GenBank accession number assigned as JF330782 (*OaN2*), JF330783 (*OaN5*), and JF330784 (*OaN6*). Northern analyses indicated that the mRNA transcripts of *OaN2* were mainly seen in ovine mesenteric lymph nodes and spleen, while *OaN5* was observed in only in mesenteric lymph nodes. In contrast, *OaN6* transcripts were detected in all tissues except for liver and heart. Western blot showed that *OaN2* protein expression level was detected in mesenteric lymph nodes, spleen, and liver, essentially consistent with that of mRNA transcripts. Immunohistochemistry analysis showed that *OaN2* protein was highly expressed in ovine mesenteric lymph nodes, moderately expressed in, and not expressed in heart, liver, and pancreas, consistent with the results of Western blotting. The cloning and expression analysis of 3 novel genes provide a basis for revealing their specificities and would be helpful to further study of their expression profile and their potential functions.

Keywords: sheep, MHC, novel gene, expression analysis

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