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論文内容の要旨

All jawed vertebrate species examined so far have major histocompatibility complex (MHC) class Ⅰ molecules that trigger cell-medieted immunity by displaying endogenously generated peptides to CD8+ cytotoxic T lymphocytes through the interaction with αβ T cell receptors (TCR). These molecules are encoded in the MHC region and known to be highly polymorphic. They are called classical class Ⅰ. There are several families that show structural homology to classical class Ⅰ molecules but have different functions, which are called non-classical class Ⅰ genes. Some of them are not involved in immune responses. They are encoded not only inside the MHC but also outside the MHC.

Recently, a novel non-classical class Ⅰ family was found near the leukocyte receptor complex (LRC) on mouse chromosome 7 in our laboratory. This newly discovered family was named *Mill* (MHC class I-like located near the LRC). The *Mill* family consists of two functional genes, *Mill1* and *Mill2*, and two pseudogenes, *Mill-ps1* and *Mill-ps2* showed sequence similarities to human *MICA* and *MICB* genes (about 40% amino acid sequence identity). This close relationship between the *Mill* and *MIC* families was also supported by phylogenetic analyses. The overall amino acid sequence identity of the α1-α3 domains between MILL1 and MILL2 was about 70%. However, each domain showed different sequence homologies. Sequence identities of the α1, α2 and α3 domains were 43%, 87%, 78%, respectively. Another notable feature of Mill1 and Mill2 was that they had an extra exon (exon2) between the signal peptide-encording exon and thea1 domain-encording exon, which wasn't observed in any class Ⅰ families.While exon2 of *Mill1* was transcribed constantly, exon2 of *Mill2* alternaitively spliced out. This extra exon of *Mill2* turned out to be part of a repetitive element, SINE-B4, but exon2 of *Mill1* was not part of such repetitive elements, which indicate that *Mill1* and *Mill2* acquired their extra exons independently. *Mill1* was expressed at rather restricted tissues such as skin and eyes neonatal mice, whereas *Mill2* was ubiquitously expressed at low levels.

To understand the evolutionary dynamics of this family and gain insights into its function, I carried out comparative analysis between mice and rats. The Mill family of rat consists of only *Mill1* and *Mill2*. There was no orthologous copy of mouse *Mill-ps1* and *Mill-ps2*. Sequence identities of MILL1 and MILL2 between mice and rats were 71% and 74% (α1-α3 domains), respectively. Apparently, this degree of sequence conservation was the least among the class Ⅰ genes that share orthologous copies between mouse and rat. However, in no domain,non-synonymous substitution rates (d_N) signicantly exceeded synonymous substitution rates(d_S); hence there was no obvious evidence for the positive selection. Interestingly, the α2 domain of mouse rat *Mill1* showed most similarity to that of mouse *Mill2* rather than rat *Mill*1. The α2 domain of rat *Mill1* showed most similarity to that of rat *Mill2*. Similarity, the α3 domains of mouse *Mill1* and rat *Mill1* showed most similarities to those of mouse *Mill2* and rat *Mill1*, respectively. The other domains and introns did not show such relationship. These observations suggested the occurrence of gene-conversion like events at the α2 and α3 domains. Polymorphism analysis revealed that most of inbred rat strains (12 of 14 strains) had Mill2 pseudogene owing to

the same single premature stop codon mutation, which indicates that the functional constraint on *Mill2* was low. Deduced exon/intron structures of mouse and rat *Mill* genes were similar but not identical The most remarkable difference was the absence of the extra exon in *Mill2*. Although the corresponding sequence to the extra exon was found at intron 1 of rat *Mill2*, no mature mRNA containing this sequence was observed in any tissues. Nevertheless the amino acid sequence of rat Mill2 was longer than that of mouse Mill2, because, in the rat, the region corresponding to the mouse exon 5 (α3 domain), intron 5, and exon 6 (CP/TM/CYT) became a single exon without any frameshift.

Computational homology searches revealed that the emergence of the *Mill* family could be traced back to the common ancestor of extant eutherian mammals and marsupials. A computational search followed by the isolation of cDNA from horse revealed that *Mill* is not a rodent specific class I family, although the horse *Mill* gene was a transcribed pseudogene. Further computational analysis revealed the existing of the *Mill* family was not found in any other species examined so far. So, it was suggested that the mill family was lost from several lineages independently, possibly owing to low functional constraints. Interestingly, *Mill* and *MIC* families tended to show reciprocal distribution in mammalian species. In other words, no species have both *Mill* and *MIC* families simultaneously, although it is not yet known about functional correlation between them. Thus, the *Mill* family is supposed to have undergone dynamic changes through mammalian evolution, which may be due to the species specific functions or overlapping function with other genes.

論文の審査結果の要旨

古典的な主要組織適合遺伝子複合体(MHC)クラスⅠ分子は、外来タンパク質の分解産物（ペプチド）をキラーT細胞に提示することにより、同細胞を活性化する。このしくみが、ウイルス感染細胞やがん細胞の排除に中心的な役割を担っているのは、周知の事実である。これを対して、非古典的MHCクラスⅠ分子（MHCクラスⅠ分子と構造的に類似しているが、他型性に乏しく、しばしばMHC領域外でコードされている）の構造と機能に関しては不明な点が多い。本博士論文は、新しい非古典的クラスⅠ遺伝子群 *Mill* (MHC class Ⅰ-like located near the leukocyte receptor complex) について、主として遺伝子レベルでの解析を行ったものであり、大別すると、1) マウスにおける *Mill* 遺伝子群の同定と解析、2) ラットとマウス *Mill* 遺伝子群の比較ゲノム解析、3) 哺乳類における *Mill* 遺伝子群の進化と起源の3部からなる。

まず、「マウスにおける *Mill* 遺伝子群の同定と解析」のセクションにおいては、既知の非古典的MHCクラスⅠ遺伝子のいずれとも異なる新しいクラスⅠ遺伝子群を発見したことが報告されている。具体的には、1) マウスMILL分子には、MILL1,MILL2と名づけられた二つのメンバーが存在する、2) 両分子ともMHC領域ではなく、第7染色体上のleukocyte receptor complex (LRC) 領域近傍でコードされている、3) MILL1、MILL2分子には各々少なくとも二つのアリルが存在する、4)既知のクラスⅠ様分子との比較では、MILLは、ヒトMICA・MICB分子と最も高い配列相似（アミノ酸レベルで約40％の相同性）を示す、5)MILLは正常成体組織にはほとんど発現されていない、などの点を明らかにしている。

次いで、「ラットとマウス *Mill* 遺伝子群の」のセクションでは、1) ラットにもマウスと同様、LRC領域に二つの *Mill* 遺伝子（*Mill1,Mill2*）が存在すること、2) ラットの多くの系統では *Mill2* 遺伝子が偽遺伝子となっていること、3) *Mill* 遺伝子群のalpha 1ドメインでは、非同義的塩基置換が同義的塩基置換に比し、有意に高頻度に認められることが報告されている。これらのデータは、*Mill* が速い進化速度を示すクラスⅠ遺伝子群であり、そのalpha1ドメインには陽性選択圧がかかっていることを示唆するものである。

「哺乳類における *Mill* 遺伝子群の進化と起源」のセクションでは、*Mill* 遺伝子群がげっ歯類に固有なクラス□遺伝子群ではなく、有袋類と真獣類の共通祖先の段階で出現したと推測される非古典的クラス□遺伝子であることが述べられている。*Mill* 遺伝子群は、ヒトを始め、いくつかの哺乳類種では失われたと考えられるが、興味深いことに *Mill* 遺伝子群を持たない種では *MICA・MICB* 遺伝子群が存在し、逆に *MICA・MICB* 遺伝子群を欠落した生物種では *Mill* 遺伝子群を保有する傾向が認められた。この現象のみは不明であるが、今後、*Mill* 分子の機能を解明する上で重要な知見と考えられる。

以上で述べたように、本博士論文には多数の重要な新知見が盛り込まれており、本論文は博士（学術）に十分値するものであると判断した。なお、「*Mill* 遺伝子群の比較ゲノム」に関連する部分は、申請者を筆頭著者として、免疫学分野における国際学術誌である *European Journal of Immunology* に掲載済みであり、「マウスにおける *Mill* 遺伝子群の同定と解析」については、申請者を第二著者として *Proceedings of the National Academy of Science(USA)* に掲載されている。