マウス肝におけるリソソーム膜糖蛋白質、LGP85/LIMP IIの同定とその性質

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Identification and Characterization of a Major Lysosomal Membrane Glycoprotein, LGP85/LIMP II in Mouse Liver

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ABSTRACT We previously have purified and characterized a major lysosomal membrane glycoprotein termed LGP85(LIMP II) in rat liver lysosomes. In this study, LGP85 in mouse liver lysosomes was identified and characterized by biochemical and molecular biological methods. Lysosomal membranes were isolated from murine liver by differential centrifugation. LGP85 was present in the lysosomal membrane fraction from mouse liver in a comparable amount to another lysosomal glycoprotein, lamp-2. Mouse LGP85(M-LGP85) from liver lysosomal membranes exhibited an M_r of 80,000 on SDS-PAGE, which is smaller by 5,000 than that of rat LGP85(R-LGP85). M-LGP85 was immunochemically detected in the extracts of brain, heart, lung, liver, and kidney. A cDNA encoding M-LGP85 was cloned from mouse liver cDNA library. The primary protein structure deduced from a nucleotide sequence of M-LGP85 cDNA indicated that M-LGP85 consists of 478 amino acids with M_r of 54,069. M-LGP85 showed 93.3 and 86.0% sequence similarities to its rat and human counterparts in amimo acids, respectively. M-LGP85 contains 11 potential N-glycosylation sites which are heavily glycosylated, resulting in the increased Mr of M-LGP85 present in the mouse liver lysosomes. It is likely that M-LGP85 traverses the lysosomal membrane twice, with an NH_2 -terminal transmembrane domain, and another hydrophobic domain near the COOH-terminus. M-LGP85 has a protruding

COOH-terminal cytoplasmic tail consisting of amino acid residues including the leucine-isoleucine sequence shown to be the lysosomal targeting signal of R-LGP85 and human LGP85(H-LGP85). The high level of expression of M-LGP85 in the lysosomal membrane, the high structural similarities among M-, R-, and H-LGP85, and the occurrence of M-LGP85 in all the mouse tissues examined suggest the essential and constitutive function of LGP85 in lysosomes.

抄録 マウス肝リソソーム画分より精製したLGP85(M-LGP85)について、その生化学的および分子生物学的性質を調べ、ラットおよびヒトLGP85(R-およびH-LGP85)と比較検討した。M-LGP85の分子量はR-LGP85の分子量よりも、約5,000小さかった。M-LGP85は肝以外にも、脳、心、肺、脾、腎に検出された。一方、M-LGP85cDNAの塩基配列から推定されるM-LGP85は分子量54,069の蛋白質であり、蛋白質の大部分はリソソーム内腔にあり、そこの11カ所にN-結合型糖鎖の結合部位が存在していた。さらに、M-LGP85はNH_2末端側とCOOH末端付近に2カ所の膜結合部位を持ち、COOH末端を含む20個のアミノ酸から成るペプチドを細胞質に突き出していた(細胞質テール)。M-LGP85はアミノ酸配列でR-LGP85とH-LGP85とそれぞれ93.3%と86%の相同性を有していた。特にLGP85の細胞内輸送に関与していると考えられている細胞質テールのアミノ酸配列は3種できわめて類似していた。以上の事実はLGP85がリソソーム膜機能の本質的な部分に関与しているものと推察される。