培養肺胞II型上皮細胞RLE-6TNにおける FITC-アルブミンのクラスリン介在型エンドサイトーシス

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Clathrin-mediated endocytosis of FITC-albumin in alveolar type II epithelial cell line RLE-6TN

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ABSTRACT : We examined mechanisms of FITC-albumin uptake by alveolar type II epithelial cells using cultured RLE-6TN cells. Alkaline phosphatase activity and the expression of cytokeratin 19 mRNA, which are characteristic features of alveolar type II epithelial cells, were detected in RLE-6TN cells. The uptake of FITC-albumin by the cells was time and temperature dependent and showed the saturation kinetics of high- and low-affinity transport systems. FITC-albumin uptake was inhibited by native albumin, by chemically modified albumin, and by metabolic inhibitors and bafilomycin A(1), an inhibitor of vacuolar H(+)-ATPase. Confocal laser scanning microscopic analysis after FITC-albumin uptake showed punctate localization of fluorescence in the cells, which was partly localized in lysosomes. FITC-albumin taken up by the cells gradually degraded over time, as shown by fluoroimage analyzer after SDS-PAGE. The uptake of FITC-albumin by RLE-6TN cells was not inhibited by nystatin, indomethacin, or methyl-beta-cyclodextrin (inhibitors of caveolae-mediated endocytosis) but was inhibited by phenylarsine oxide and chlorpromazine (inhibitors of clathrin-mediated endocytosis) in a concentration-dependent manner. Uptake was also inhibited by potassium depletion and hypertonicity, conditions known to inhibit clathrin-mediated endocytosis. These results indicate that the uptake of FITC-albumin in cultured alveolar type II epithelial cells, RLE-6TN, is mediated by clathrin-mediated but not by caveolae-mediated endocytosis, and intracellular FITC-albumin is gradually degraded in lysosomes. Possible receptors involved in this endocytic system are discussed.

抄録　培養肺胞Ⅱ型上皮細胞（RLE-6TN細胞）を用いてFITC標識アルブミン（FITC-ALB）の取り込み機構を検討した。FITC-ALBの取り込みは、時間及び温度依存的であり、飽和のある輸送システムであることがわかった。共焦点走査型顕微鏡を用いた解析により、蛍光は部分的にライソゾームに局在することが明らかとなった。FITC-ALBはライソゾーム中で徐々に分解していくことも見出した。また、取り込み阻害剤を用いた実験により、FITC-ALBのRLE-6TN細胞への取り込みはクラスリン介在型エンドサイトーシスであることを示した。

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