様式 C-19

科学研究費補助金研究成果報告書

平成　23 年　6 月　3 日現在

機関番号：14202
研究種目：基盤研究（C）
研究期間：2008～2010
課題番号：20591058
研究課題名（和文）　ヒト肝ＳＲＥＢＰ－１ｃ遺伝子発現を調節する新しい核蛋白の同定とその機能解析
研究課題名（英文）　Identification and characterization of a new factor regulating SREBP-1c gene in liver.

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研究成果の概要（和文）：　RBMX とそれに結合する調節蛋白群 SAFB が、SREBP-1c の発現を転写レベルで調節していることを示してきた。さらに RBMX を中心としたこのシステムがヒト由来の培養肝細胞においても働いている事を明らかにできた。新たに我々が RBMX と結合する蛋白として yeast two–hybrid system を用いて見いだした hypothetical protein LOC 673353 が実際にユニークな遺伝子として mRNA および蛋白として発現し、SREBP-1c 遺伝子転写促進活性を持っている事を明らかにした。

研究成果の概要（英文）：We have shown that RBMX and its binding protein SAFB regulate the transcriptional activity of SREBP-1c gene. Furthermore, we have identified a new gene, hepothetical protein LOC 673353, really expressing as mRNA and protein regulating SREBP-1c gene.

交付決定額

（金額単位：円）

	直接経費	間接経費	合　計
2008 年度	1,500,000	450,000	1,950,000
2009 年度	1,400,000	420,000	1,820,000
2010 年度	700,000	210,000	910,000
年度			
年度			
総　計	3,600,000	1,080,000	4,680,000

研究分野：医歯薬学
科研費の分科・細目：内科系臨床医学・代謝学
キーワード：脂質合成、転写調節、栄養素

１．研究開始当初の背景

メタボリックシンドロームの成因として、過剰な栄養素の摂取により肝臓での脂肪合成が亢進することが重要である。肝臓での脂肪合成を担う脂肪合成酵素群は転写因子 Sterol Regulatory Element Binding Protein (SREBP)-1 によりその発現が調節されている。我々はラットやマウスにおいて高果糖食が肝臓での SREBP-1 の発現を亢進させてメタボリックシンドローム症状を誘導することを報告した(文献 18, Nagai Y et al. Am J Physiol 2002, Sinozaki K et al. Circ Res 2000)。 しかしながら、純系マウスでは系統によって果糖食による症状発現が異なり、それが肝臓での SREBP-1 の発現の差異により説明できること、また、その反応の差異に SREBP-1c のプロモータ領域の−468 番塩基の変異の有無が重要であることを報告した(文献 10)。さらに、我々はこの遺伝子領域を介して SREBP-1c 遺伝子の転写調節に関与する蛋白を抽出し、TOF-mass 解析した結果、RNA-binding motif protein on X

chromosome(RBMX)を同定した(文献 1)。RBMX の肝細胞への過剰発現はマウス SREBP-1c プロモーター活性を 5-7 倍増加させた。しかしながら、RBMX は SREBP-1c プロモーターに直接結合できないことが判明し、何らかのアダプター蛋白を介して SREBP-1c プロモーターを調節すると予想できた。

２．研究の目的

RBMX を bait とした Two-hybrid screening ならびに SREBP-1c のプロモータ領域の-468 番塩基付近の DNA 配列を bait とした One-hybrid screening を行い、アダプター蛋白の検索を行ない得られた hypothetical protein LOC 673353 が実際にユニークな遺伝子として mRNA および蛋白として発現し、SREBP-1c 遺伝子転写促進活性を持っている事を明らかにする

３．研究の方法

1) ゲルシフト解析： LOC 673353 をクローニングし、in vitro 発現システムを用いて目的遺伝子の蛋白発現を行う。得られた蛋白を用いて RBMX 反応部位の DNA をプローブとしてゲルシフト解析を行い候補遺伝子が目的の DNA 配列を特異的に認識して結合することを確認する。

2) GST プルーダウン解析： 候補遺伝子とグルタチオン S-トランスフェラーゼ(GST)の融合蛋白を大腸菌を用いて合成し、ヒト肝細胞の抽出サンプルと共孵置し、グルタチオン結合ビーズを用いて候補遺伝子と結合している蛋白のウェスタンブロット解析を行う。

3) 候補遺伝子による SREBP-1c プロモーター活性化の検討： 候補遺伝子を強制発現ベクターにクローニングし、ヒト 1.6kbSREBP-1c プロモーターレポーターや RBMX 反応部位を変異させた SREBP-1c プロモーターレポーターとともに HepG2 細胞に導入しルシフェラーゼ解析を行う。さらに、候補遺伝子の siRNA を用いて内因性の分子を抑制した状態で RBMX 強制発現下にヒト 1.6kbSREBP-1c プロモーター解析を行う。

４．研究成果

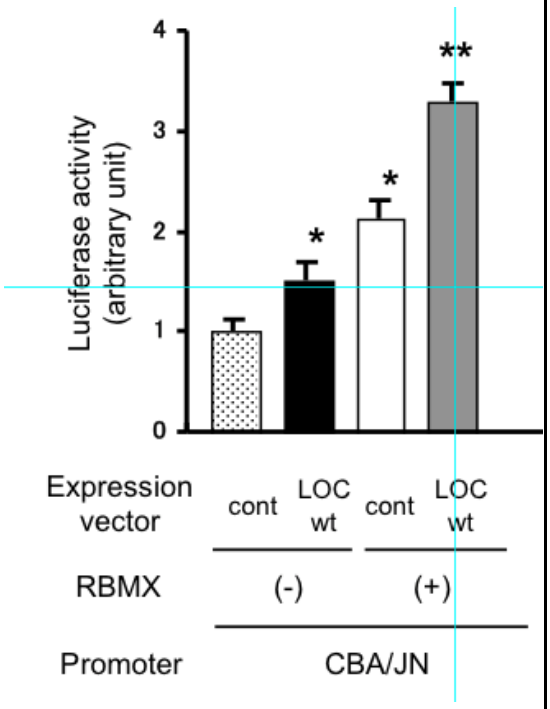
(1) *LOC673353 recognizes the SNP at -468 bp of SREBP-1c promoter*- To confirm the binding of LOC673353 to the upstream region of the SREBP-1c gene, we performed EMSA using the labeled oligonucleotides extending from -477 to -458 bp of SREBP-1c promoter region in the CBA/JN or DBA/2N strain as a probe. Purified GST-fusion LOC673353 protein (GST-LOCwt) was incubated with either CBA/JN or DBA/2N probe. The DNA-protein binding was detected when GST-LOCwt protein was incubated with CBA/JN probe but the signal was markedly suppressed when the GST-LOCwt protein was incubated with DBA/2N probe. The binding of LOC673353 to CBA/JN probe was lost when the region identified by one-hybrid analysis was deleted from LOC673353 (GST-LOCMA). Furthermore, the binding of LOC673353 protein (GST-LOCwt) to CBA/JN probe was specific since the binding to the other consensus sequence such as Oct-1 was not observed.

(2) *Interaction between LOC673353 and RBMX*- To investigate the interaction between LOC673353 and RBMX, we performed the GST pull-down assays. RBMX produced in rabbit reticulocytes (pTNT-RBMX) was recovered by GST-LOCwt but not by unfused GST, suggesting the existence of a direct interaction between LOC673353 and RBMX. This interaction disappeared when the region identified by two-hybrid analysis in LOC673353 was deleted (GST-LOCMB), but not when the region identified by one-hybrid analysis in LOC673353 was deleted (GST-LOCMA). Conversely, the interaction was enhanced when the N-terminal region was deleted (GST-LOCMC).

(3) *The mRNA and protein expression of LOC673353 in the liver and other tissues*- To clarify the gene expression of LOC673353, we investigated the mRNA expression of LOC673353 in the mouse liver. At first, we performed Northern blot analysis of LOC673353 using poly(A)$^+$ RNA prepared from mouse liver tissues. Several bands were detected using full-length LOC673353 cDNA probe. Next, we prepared cDNA of LOC673353 using reverse transcription from mouse liver RNA with oligo(dT) primer followed by PCR. The nucleotide of this PCR product at 310 bp from the starting point was adenine, which is identical to that found in LOC673353 but different from 18S rRNA (which is guanine). In addition, we examined the expression of mouse LOC673353 using reverse transcription from poly(A)$^+$ RNA and total RNA prepared from mouse liver

followed by quantitative real-time PCR. The amount of PCR product of LOC673353 from poly(A)$^+$ RNA was increased compared with that from total RNA, while the amount of PCR product of 18S rRNA from poly(A)$^+$ RNA was decreased as compared with that from total RNA.

(4) *LOC673353 activates the SREBP-1c promoter in hepatocytes*- To determine whether LOC673353 functionally interacts with the SREBP-1c promoter, the Fao cell line, a rat hepatoma cell line, was cotransfected with the expression construct for LOC673353 (pIRES2GFP-LOCwt) and the luciferase reporter carrying the SREBP-1c promoter of CBA/JN (pGL3-CBA/JN) or DBA/2N (pGL3-DBA/2N) strain. Overexpression of LOC673353 significantly increased the activity of CBA/JN-SREBP-1c promoter in the presence or absence of RBMX overexpression (Fig).



The effect of overexpression of LOC673353 on the DBA/2N-SREBP-1c promoter in the presence or absence of RBMX overexpression, however, was blunted. The mutant LOC673353 (LOCMA) lacking the region identified by one-hybrid analysis did not increase the activity of CBA/JN-SREBP-1c promoter. Corresponding to the result of enhanced interaction between RBMX and GST-LOCMC, the effect of the overexpression LOCMC on the CBA/JN-SREBP-1c promoter was increased as compared with that of the overexpression of wild-type LOC673353.

(5) *Effect of RNA interference on SREBP-1c promoter activity and expression*- To examine the influence of the decreased expression of LOC673353 on CBA/JN-SREBP-1c promoter activity, we used siRNAs. Although the inhibition of LOC673353 by siRNA did not affect the basal CBA/JN-SREBP-1c promoter activity, the activity of the SREBP-1c promoter induced by overexpression of RBMX was reduced by 50% in the cells cotransfected with the LOC673353 siRNA, but not in those contransfected with control siRNA. In addition, the band detected with the labeled oligonucleotides at the region from -477 to -458 bp of CBA/JN-SREBP-1c promoter in EMSA was reduced when the expression of LOC673353 was knocked down with siRNA. However, the decreased expression of LOC673353 associated with siRNA did not affect the binding activity of the other transcription factor, Oct-1.

(6) *LOC673353 directly binds to the promoter of SREBP-1c gene and RBMX in the mouse liver*- To confirm the binding of LOC673353 to the upstream region of the SREBP-1c gene in the mouse liver, we performed ChIP assay. Primers were designed for detecting the regions containing the mouse SREBP-1c promoter around -468 bp. The ChIP assay clearly showed for the liver, LOC673353 binding to this region of SREBP-1c gene. In addition, for confirmation of the binding of LOC673353 to RBMX in the liver, immunoprecipitation assay was performed. Anti-RBMX antibodies were used to immunoprecipitate proteins from mouse liver, and the immunoprecipitated proteins were analyzed by Western blot analysis using LOC673353 antibody. The binding signal was detected in the immunoprecipitated protein by using RBMX antibody but was not detected with control antibody.

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６．研究組織

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