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Original Research Article

lncRNA profiling to elucidate the metabolic mechanism of green tea extract on weight loss in mice

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Abstract

Purpose: *To understand the effects of green tea extract on weight loss at the gene level using long non-coding RNA (lncRNA) expression profiles.*
Methods: *lncRNA expression signatures in rats fed two different diets were determined by analyzing previously published gene expression profiles in Gene Expression Omnibus (GEO). The lncRNAs specific to rats in a particular dietary group were confirmed using an additional autonomous dataset. LncRNA expression profiles were compared to explore the underlying mechanisms of green tea extract on weight loss.*
Results: *Three lncRNAs (Gm38399, F730035P03Rik, and 5033430I15Rik) that may be the targets of green tea and that may play crucial roles in the lipid-lowering effects of green tea were identified. Using functional annotation databases, two of the targets of two of the lncRNAs were identified as Nav1 and Atxn1.*
Conclusion: *Based on annotation databases, green tea extract may affect metabolic processes in adipocytes by regulating the lncRNAs GM38399 and 5033430I15Rik that modulate their cis-regulatory target genes Nav1 and Atxn1, respectively. Nav1 and Atxn1 may then regulate trans-regulatory lncRNAs.*

Keywords: *Green tea, Epigallocatechin gallate, Obesity, Weight loss, LncRNA profiling*

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INTRODUCTION

Obesity is associated with cardiovascular disease, diabetes mellitus, and cancer and stems from a combination of environmental effects and genetic factors. Obesity is gaining attention worldwide as a major contributor to poor health, thus there is a need to develop anti-obesity therapies and treatments [1]. Due to the complexity of obesity, traditional oriental medicines (TOMs) are often used as alternative treatments for obesity. In contrast to conventional drugs, TOMs are advantageous because they are multi-component and multi-targeted natural products [2].

Green tea, which has high amounts of catechin polyphenols and caffeine, has been used as a "medicine" to reduce body weight [3]. Green tea

has been shown to promote energy expenditure, fat oxidation, and weight loss, and these functions are predominantly attributed to the catechin polyphenols in green tea [4]. The four main catechins in green tea are epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) [5]. Because EGCG is the most abundant catechin (~50–75% of total catechins) in green tea, EGCG is thought to be responsible for the health-promoting effects of green tea, including weight loss [6]. It has been suggested that consumption of EGCG augments weight loss by diet-induced thermogenesis [7].

A conventional method to evaluate the impact of EGCG on weight loss involves comparing the weights of animals consuming diets with or without EGCG. However, this method provides limited information because conclusions can only be made at the level of the individual animal [8]. Non-coding RNAs (ncRNAs) are gaining attention due to their involvement in posttranscriptional regulation. Long ncRNAs (lncRNAs), a new class of regulatory RNA molecules, have been investigated as possible biomarkers and therapeutic targets for various diseases [9]. In this study, the mechanistic function of EGCG on weight loss in rats was investigated at the gene level using lncRNA expression profiles.

The aim of this study was to profile the lncRNA signatures in rats fed two different diets using previously published green tea gene expression profiles in Gene Expression Omnibus (GEO) [9]. The lncRNAs specific to rats in a particular dietary group were confirmed using an additional autonomous data set. Then, the lncRNA expression profiles were compared to explore the underlying mechanisms of green tea extract on weight loss. Three lncRNAs were found that associated with the weight loss function of green tea extract, and these lncRNAs were evaluated for function by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses.

METHODS

GEO gene expression data

GEO datasets were screened for microarrays related to the efficacy of green tea extract on fat metabolism. The GEO accession series GSE71586 matched the screening conditions and was used in this study. All of the data sets in the microarray were acquired with the Illumina HiSeq 2500 platform and the data underwent logarithmic transformation for homogeneity of differences. The dataset contained data from the normal diet (control) group, the high-fat diet group, and the high-fat + 3% green tea extract diet group (tea group). The high-fat diet group was given a high-fat diet for 12 weeks, the tea group was given the high-fat diet + 3% green tea extract for 12 weeks, and the control group was given a maintenance diet for 12 weeks. The probe Ensemble IDs were annotated with gene names.

LncRNA extraction

Probes with Ensemble gene IDs were imported into the Ensemble Online database, and probes labeled “lncRNA”, “processed_transcripts”, “non-coding”, or “misc_RNA” were reserved. Pseudogenes, rRNAs, microRNAs, or other short RNAs (tRNAs, snRNAs, and snoRNAs) were refined via the “transcript type” option in the Ensemble Online dataset. A total of 208 annotated lncRNA transcripts were obtained.

Analysis of differentially expressed lncRNAs

R software version 3.4.1 Limma package for Windows was used to compare differentially expressed lncRNAs in the high-fat diet group versus the control group and the high-fat diet group versus the tea diet group. The analysis conditions included a false discovery rate (FDR) of < 20%, fold change ≥ 2, and a p value < 0.01.

Prediction of lncRNA targets and functional enrichment

The targets of differentially expressed lncRNAs were predicted by assessing cis/trans effects. To evaluate cis effects, protein-coding genes 10 kb upstream or downstream of lncRNAs were chosen and assessed for co-expression with lncRNAs. To evaluate trans effects, expression levels of differentially expressed lncRNAs and protein-coding genes were evaluated for co-expression. Pearson’s correlation coefficients of $r > 0.8$ or < -0.8 and $p < 0.05$ indicated co-expression. Co-expression networks were visualized by Cytoscape software (v3.4.1). Target genes were annotated based on the GO database and the KEGG database was used for pathway analyses of target genes. GO terms and KEGG pathways with $p < 0.05$ were considered significantly enhanced.

Statistical analysis

The data were collected using Microsoft Office Excel Version 2013 for Windows and analyzed statistically using SPSS 22.0. Continuous data were reported as mean ± SD. The Student’s t

test was used to compare changes among individual groups. The Pearson correlation analysis was performed to evaluate the relationships between the lncRNAs and the differentially expressed genes. Unless otherwise indicated, $p < 0.05$ indicated statistical significance.

RESULTS

Dataset characteristics

The original microarrays contained 12 specimens from four groups: the control group, the high-fat diet group, the tea group, and the Taeeumjowuitang (TJ) group (n = 3/group). However, the TJ group was not used in this study. Following logarithmic transformation, the data were determined to be homogenous and met the conditions for analysis. Regarding the lncRNA expression profiles, GSE71586 contained 23,474 probes corresponding to 22,733 genes. Overall, 208 probe sets with Ensemble IDs that corresponded to 208 lncRNAs were identified (Figure 1).

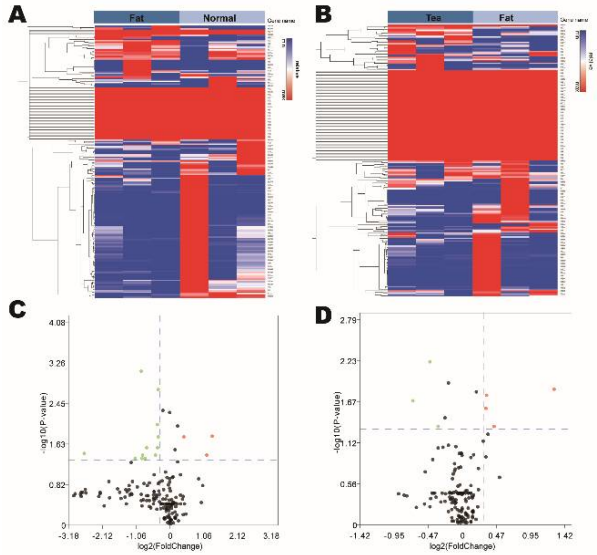

Figure 1: A, B: Differentially expressed lncRNA probes in the high-fat group vs. the normal diet group (A) and the tea group vs. the high-fat group (B). C, D: Scatter plots of gene expression in the high-fat group vs. the normal group (C) and the tea group vs. the high-fat group (D)

Differential lncRNA expression

Following Limma analysis, 14 differentially expressed lncRNAs were identified when the high-fat diet group was compared to the control group and 7 differentially expressed lncRNAs were identified when the tea group was compared to the high-fat diet group. Three of the lncRNAs were identified in both of these comparisons (Figure 2).

The lncRNAs Gm38399 and F730035P03Rik were overexpressed and 5033430I15Rik was downregulated in the high-fat group when compared to the control group. However, Gm38399 and F730035P03Rik were downregulated and 5033430I15Rik was upregulated in the tea group when compared to the high-fat normal group (Figures 1 and 2) indicating that Gm38399, F730035P03Rik, and 5033430I15Rik may be targets of green tea extract and that these lncRNAs may play important roles in the lipid-lowering effects of green tea extract.

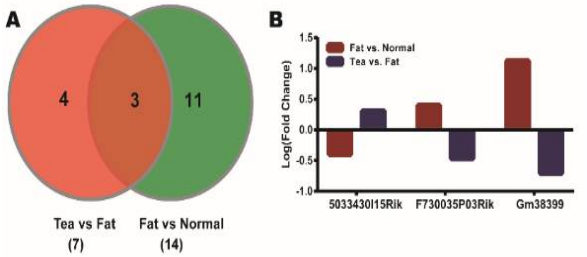

Figure 2: A. Quantification of gene expression in the high-fat group vs. the normal group and the tea group vs. the high-fat group. B. Overexpression and downregulation of genes in the high-fat group vs. the normal diet group and the tea group vs. the high-fat diet group

Trans-regulatory patterns and lncRNA functions

The trans-acting modes of the lncRNAs were analyzed to identify lncRNA target genes involved in regulation of lipid metabolism. Pearson correlation analysis showed 613 correlations and 602 genes when the lncRNA-gene correlation coefficient ≥ 0.8. Ten transactivated genes associated with 5033430I15Rik, 530 transactivated genes associated with Gm38399, and 74 transactivated genes associated with F730035P03Rik (Supplementary Table S1). The resulting co-expression network is shown in Figure 3.

LncRNA co-expression genes were also examined using the database for annotation, visualization, and integrated discovery (DAVID) [10]. The top 10 altered GO terms in the comparison groups were categorized by biological process (BP), cellular component (CC), and molecular function (MF) and were scored for fold-enrichment (Figure 4). The most enriched BP terms involved cellular signaling and the immune response: *'signal transduction* (GO:0007165)*', 'immune system process*

(GO:0002376)*'* and *'inflammatory response* (GO:0006954)'. The most enriched CC terms involved the cell membrane: *'membrane', 'cytoplasm', 'integral component of membrane',* and *'plasma membrane'.* The most represented MF terms were *'protein binding', 'metal ion binding'*, and *'ATP binding*'. The top 30 most enriched KEGG pathways are shown in Figure 5. The most enriched KEGG pathways included *pathways in cancer* (mmu05200)*, proteoglycans in cancer* (mmu05205)*, PI3K-Akt signaling pathway* (mmu04151), and *osteoclast differentiation* (mmu04380).

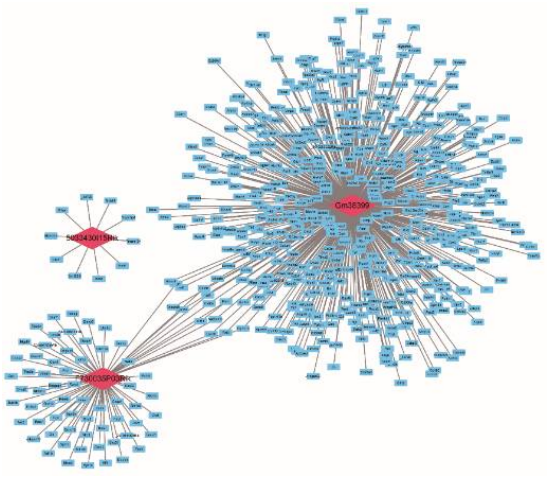

Figure 3: Co-expression networks generated by gene co-expression analysis. Red, lncRNA; blue, protein-coding gene; line, correlative association

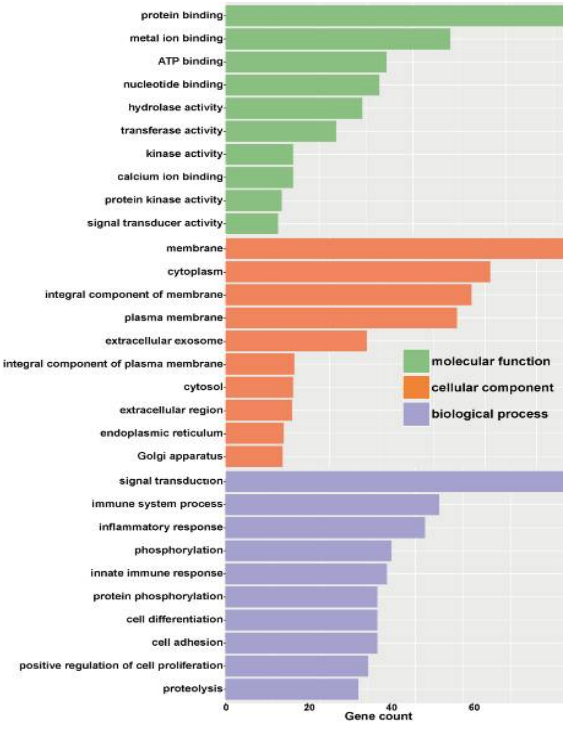

Figure 4: GO analysis of genes co-expressed with lncRNAs

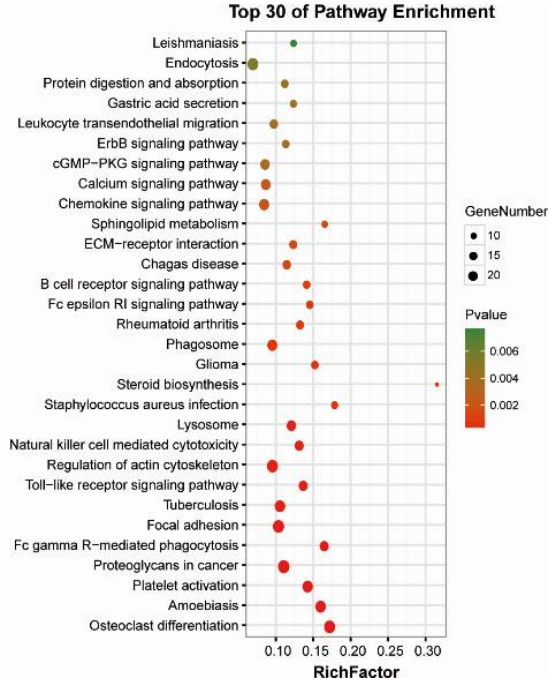

Figure 5: KEGG Pathway analysis of genes co-expressed with lncRNAs.

Cis-regulatory patterns and lncRNA functions

Four genes upstream and downstream of the 10 kb lncRNA regions that met the conditions of lncRNA cis-regulation were identified using the BLAT program of the UCSC genome browser. Nav1 and Gm4739 were identified as targets of GM38399, Olfr521 was identified as a target of F730035P03Rik, and Atxn1 was identified as a target of 5033430I15Rik (Figure 6).

GO analysis of the target genes showed that Nav1 was enriched in *microtubule bundle formation* (GO: 0001578)*, neuron migration* (GO: 0001764)*, multicellular organism development* (GO: 0007275)*, nervous system development* (GO: 0007399), and *cell differentiation* (GO:0030154). Atxn1 was enriched in *brain development* (GO: 0007420), *lung alveolus development* (GO: 0048286), *nuclear export* (GO: 0051168), and *positive regulation of glial cell proliferation* (GO: 0060252). There was no functional annotation information for the other target genes.

DISCUSSION

Previous studies have shown that EGCG is the most abundant catechin found in green tea and that other catechins, including ECG, EGC, and EC, are also found in green tea, but in relatively minor quantities.

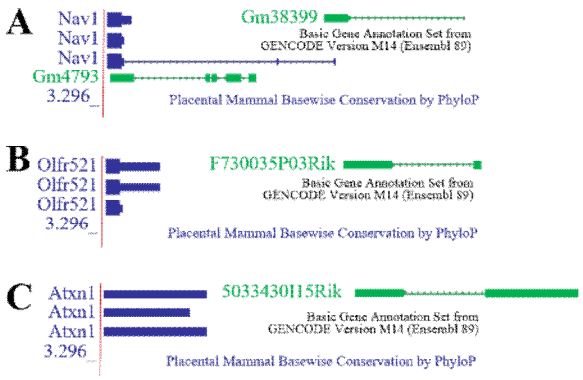

Figure 6: GM38399 (A), F730035P03Rik (B), and 5033430I15Rik (C) displayed on UCSC's genome browser

The residual solids of green tea contain caffeine, theanine, theaflavins, theorubigins, quercetin, as well as supplementary phenolics, such as gallic acid and chlorogenic acid [11]. Green tea has been shown to play roles in numerous biological activities, for example, green tea has been shown to reduce body weight and to promote maintenance of a healthy weight [12]. EGCG has also been shown to inhibit metabolic syndrome and fatty liver disease [13]. EGCG has been shown to be a mitochondria-targeted molecule that blocks mitochondrial deterioration and contributes to maintenance of normal mitochondrial metabolism [14]. When used as a medicine along with some chemotherapeutic drugs, EGCG improved the antitumor effects of the drugs [15]. In addition, EGCG inhibited teleocidin-induced tumor promotion [16]. In another study, EGCG accelerated glucose uptake and induced the IRS-1/Akt/GLUT2 signaling pathway illustrating the protective effects of EGCG on metabolic misalignment [17]. Further, EGCG may protect neurons against regressive apoptosis and may improve neuronal survival time after nerve transection [18]. Based on the literature, there are many functions that may be attributed to EGCG.

In this study, three lncRNAs (Gm38399, F730035P03Rik, and 5033430I15Rik) that may be targets of green tea extract and that may play crucial roles in the lipid-lowering effects of green tea extract were identified. After analysis of the cis-regulatory patterns and functions of these lncRNAs, it appears that green tea may affect metabolic processes in adipocytes via the cis-regulatory target genes Nav1 and Atxn1, which may then affect the trans-regulatory lncRNAs GM38399 and 5033430I15Rik. Once Olfr521, the target gene of lncRNA F730035P03Rik, is functionally annotated, the effect of lncRNA F730035P03Rik on lipid metabolism may be elucidated and the mechanism of green tea extract on adipocyte metabolism may be revealed.

A recent study reported that dietary supplementation with EGCG led to a dose-dependent reduction in mortality, however there was no effect on cognitive or muscle function [19]. Another study reported that a high-fat diet supplemented with EGCG led to a dose-dependent reduction in body weight and fat gain [20]. Treatment of mice with 0.32% dietary EGCG (a high dose) led to a reduction in the indicators of Type II diabetes and the severity of obesity-associated fatty liver disease [21]. However, a low dose of EGCG was also found to be effective [22].

CONCLUSION

Dietary supplementation with green tea extract has been shown to increase weight loss. In this study, two lncRNA target genes, Nav1 (target of lncRNA GM38399) and Atxn1 (target of lncRNA 5033430I15Rik) have been identified. Future studies on Nav1 and Atxn1 may reveal the molecular effects of green tea extract on weight loss.

DECLARATIONS

Acknowledgement

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Conflicts of interest

No conflict of interest associated with this work.

Author contributions

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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