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Mechanisms of Selenoprotein P translation regulation by long noncoding RNA

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Abstract

Selenium (Se) is one of the essential trace elements in the body. Se is present in proteins in the form of selenocysteine (Sec), in which the sulfur of cysteine (Cys) is replaced by Se. These proteins are referred to as selenoproteins. There are 25 selenoproteins in the human genome, and they play important roles in various physiological functions, including as an antioxidant and in the synthesis of thyroid hormones. Sec is inserted into selenoproteins using the Sec insertion sequence (SECIS), which is located in the 3′ untranslated region. We have identified an antisense long noncoding RNA, *CCDC152*, which binds mRNA of selenoprotein P (SELENOP), one of the plasma selenoproteins. *CCDC152* inhibits the binding of SECIS binding protein 2 (SBP2), which is a key protein for selenoprotein translation, to SECIS by direct interaction with *SELENOP* mRNA. Inhibiting the formation of the SBP2 and SECIS complex by *CCDC152* reduces the binding of ribosomes to *SELENOP* mRNA and suppresses the translation step of SELENOP. As a result, *CCDC152* causes a decrease in SELENOP protein levels independent of *SELENOP* mRNA levels. No impact was observed on the protein and mRNA expression levels of other selenoproteins. This review describes the mechanism of SELENOP protein suppression by *CCDC152*.

Keywords: Selenoprotein, SELENOP, SECIS, long noncoding RNA, antisense RNA

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1. Introduction

Selenium transport protein selenoprotein P (SELENOP) contains the essential trace element selenium (Se) in the form of selenocysteine (Sec), which is an amino acid in which the sulfur of cysteine is replaced by Se. It has been reported that SELENOP is increased in the blood of patients with type 2 diabetes mellitus (T2DM). Excess SELENOP induces insulin resistance and impairs insulin secretion, resulting in aggravation of T2DM [1,2]. Therefore, SELENOP is expected to become a new therapeutic target of T2DM.

Selenoproteins, including SELENOP, are synthesized by a unique translation mechanism. In the 3′ untranslated region (UTR) of the mRNA encoding selenoprotein, there

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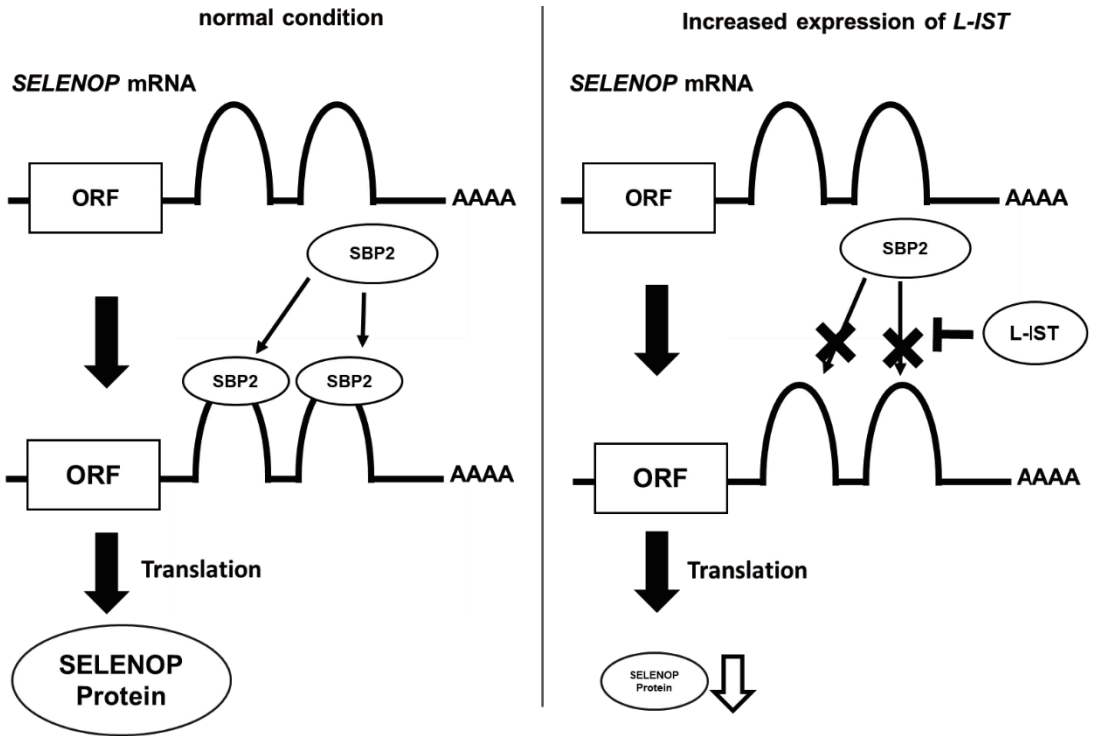

Figure Schematic diagram of the mechanism of SELENOP translation suppression by CCDC152/L-IST in hepG2cells. *CCDC152/L-IST* specifically suppresses SELENOP translation by inhibiting the binding of SBP2 to SECIS of SELENOP mRNA.
ORF: Open Reading Frame.
SBP2: SECIS Binding Protein 2.

is a Sec insertion sequence (SECIS) forming a stable loop structure. Sec-tRNAs, specific elongation factor eEFSec, and SECIS binding protein 2 (SBP2) bind to SECIS and form a complex. This complex allows for the insertion of Sec into the UGA codon, which is usually recognized as a termination codon [3]. In the process of analyzing the SECIS sequence, we identified a gene of unknown function, *coiled-coil domain-containing protein 152* (*CCDC152*), which has a complementary sequence to the SECIS-containing 3′ UTR of *SELENOP* mRNA. The overexpression of *CCDC152* in human hepatocellular carcinoma-derived HepG2 cells expressing SELENOP causes a decrease in protein levels without altering *SELENOP* mRNA levels. Since *CCDC152* is mainly found in the nucleus, the CCDC152 protein was not detectable when overexpressed in HEK293 cells, which do not express *CCDC152*. In addition, *CCDC152* RNA binds to *SELENOP* mRNA; hence, it is possible that CCDC152 functions as an RNA. In HepG2 cells overexpressing *CCDC152*, the binding of *SELENOP* mRNA to ribosomes and SBP2 was decreased [4]. Based on these functions, we named *CCDC152* a *long noncoding RNA inhibitor of selenoprotein P translation* (*L-IST*) (**Figure**).

In this review, we describe the translation mechanism of selenoprotein in the presence of SECIS and explain the action of *L-IST* that we found. We also describe *L-IST* as a new therapeutic target for reducing the increase of T2DM.

2. The mechanisms of selenocysteine insertion

Se deficiency is known to cause severe cardiomyopathy and increase the incidence of cancer [5,6]. There are 25 selenoproteins in the human genome, and they play important roles in various physiological functions. Selenoproteins include antioxidant proteins, such as glutathione peroxidase (GPx) and thioredoxin reductase (TrxR), and proteins related to thyroid hormone synthesis, such as iodothyronine deiodinase. Thus, selenoproteins play an important role in the protection of cells from oxidative stress and in the process of energetic metabolism. The insertion of Sec into proteins occurs during the translation phase, and Sec has also been called the “21st amino acid that can be

translated" [7]. The complex of SBP2 and Sec-tRNASec binds to a hairpin structure called SECIS, which is essential for Sec translation, and Sec is inserted into the UGA codon [8]. The strength of the binding between SECIS and SBP2 depends on the 3D structure of SECIS [9]. Therefore, it has been suggested that the efficiency of selenoprotein translation is dependent on the 3D structure and not the SECIS sequence [9].

3. Identification and functional analysis of *CCDC152*

We compared the SECIS sequences of mRNAs encoding 25 human selenoproteins in SelenoDB 2.0 (http://selenodb.crg.eu) [10] with the sequences of all transcripts using BLAST analysis. We found a novel gene, *CCDC152*, containing an antisense sequence to the SECIS sequence of the SELENOP. *CCDC152* contains a part of the coding sequence of SELENOP and an antisense sequence against the 3′ UTR region containing the SECIS sequence, and both sequences were completely complementary to each other. The results of the homology analysis of *CCDC152* show that it is present from fish to mammals.

Analysis of *CCDC152* expression levels in various cultured cells revealed that *CCDC152* was expressed in human neuroblastoma SH-SY5Y cells, glioma U87MG cells, and human T lymphocytoma Jurkat cells, but only slightly in hepatocarcinoma-derived HepG2 cells, which express and secrete SELENOP. Comparison of expression levels in various tissues in mice revealed that *SELENOP* was expressed mainly in the liver, small intestine, and kidney and that *CCDC152* was most highly expressed in the testes and also expressed in the liver, kidney, and white fat tissue. In high-fat, high-sucrose dietinduced diabetic model mice, there was an increase in *SELENOP* expression in the liver, while *CCDC152* in the liver tended to be decreased.

CCDC152 has a complementary sequence to *SELENOP* mRNA; therefore, we overexpressed *CCDC152* in HepG2 cells expressing SELENOP and examined its effect on SELENOP protein expression. We observed a reduction in SELENOP protein levels and no change in mRNA levels. The overexpression of *CCDC152* and *SELENOP* mRNA in HEK293 cells, which express neither *CCDC152* nor *SELENOP* mRNA, also caused a decrease in SELENOP protein levels without a change in mRNA levels, as observed in HepG2 cells. Hence, the mRNA level–independent decrease in SELENOP protein levels by *CCDC152* was not specific to a particular cell type. Next, we examined the effect of CCDC152 on other selenoproteins. There were no changes in the protein and mRNA levels of selenoproteins such as GPx4 and TrxR1 other than SELENOP, suggesting that *CCDC152* specifically affects the amount of SELENOP protein and mRNA.

There are more than 200 members of the CCDC protein family. It has been reported that CCDC80 and CCDC134 inhibit the activity of ERK [11, 12] which protein suppresses SELENOP transcription by promoting FoxO3a translocation out of the nucleus. Therefore, CCDC80 and CCDC134 may enhance the transcription of SELENOP. On the other hand, the suppression of SELENOP protein expression by *CCDC152* overexpression is not involved in transcriptional regulation. Therefore, it is likely that SELENOP protein levels are regulated by a different mechanism than the previously known CCDC protein family. Because of the presence of an open reading frame (ORF) between bases 71 and 835 in the 5′ region of *CCDC152*, we investigated whether the CCDC152 protein or RNA could reduce the protein content of SELENOP. To validate protein translation from the ORF, we inserted an HA-tag at the 3′ end of the ORF. However, western blotting with an antibody against the HA-tag did not detect the CCDC152 encoding protein. Next, we analyzed the effect of the *CCDC152* deletion mutant on the SELENOP protein. The *CCDC152* (Δ500) mutant, in which up to 500 bases were deleted from the 5′ end, including the putative initiation ATG codon, retained the ability to suppress SELENOP protein expression. However, the *CCDC152* (Δ600) mutant, in which up to 600 bases were deleted from the 5′ end, did not have the same suppressive effect. Therefore, the SELENOP-reducing effect of *CCDC152* is possibly through its action not as a protein but as an RNA.

Next, we focused on the translation step, with particular emphasis on the step involved in the binding of mRNA to ribosomes. Our results showed that CCDC152 exerts an inhibitory effect on protein synthesis in a manner independent of mRNA levels. Polysome analysis was performed to evaluate the binding of *SELENOP* mRNA to ribosomes. We found that the number of ribosome-bound *SELENOP* mRNAs was markedly diminished in *CCDC152*-overexpressing HepG2 cells. In contrast, *GPx4* mRNA, another selenoprotein mRNA, did not show a significant

change in its binding to ribosomes when *CCDC152* was overexpressed.

Subsequently, we investigated the binding of *SELENOP* mRNA to SBP2, which is an essential factor for Sec insertion via SECIS, using an RNA pull-down assay. We found that the overexpression of *CCDC152* reduced the binding affinity of *SELENOP* mRNA to SBP2. Thus, these results showed that *CCDC152* inhibits the translational step of *SELENOP* mRNA, especially the binding of SBP2 to SECIS. Based on these functions, we named *CCDC152* a long noncoding RNA inhibitor of selenoprotein P translation [4].

4. Epigallocatechin gallate increases *L-IST* expression and decreases SELENOP protein

It has been reported that elevated blood SELENOP levels are associated with T2DM [1] and pulmonary hypertension [13]. The development of a method to decrease SELENOP would be a novel therapeutic strategy for those diseases. Therefore, we searched for substances that increase *L-IST*. We found that epigallocatechin gallate (EGCg), which is known as the main ingredient of green tea with an antidiabetic effect, increased *L-IST* and decreased SELENOP protein levels without changing *SELENOP* mRNA levels. Based on these results, we administered EGCg to mice and found that *L-IST* was increased in the liver of EGCg-treated mice, but *SELENOP* mRNA levels were unchanged. Mice with increased *L-IST* by EGCg also had decreased blood levels of SELENOP and blood glucose levels, indicating that *L-IST* could be a promising therapeutic candidate agent for T2DM treatment [4].

5. Translation regulation mechanisms by noncoding RNAs

Most of the intergenic regions in the genome have been called "junk regions," which are presumed to lack any functional significance. However, in recent years, many noncoding RNAs have been found in these junk regions, and their functions have been clarified. The analysis of noncoding RNAs involved in the regulation of protein quantity has been a prominent area of research.

Protein reduction by short RNAs of approximately 20 mer, such as siRNA and miRNA, is mediated by the AGO family protein [14]. In contrast, different mechanisms of translation regulation by long noncoding RNAs have been reported for different types of RNA. *Antisense BASE-1* has been demonstrated to increase BASE-1 protein levels by causing mRNA stabilization [15]. Long noncoding RNAs containing SINEB2 sequences promote the translation of target mRNAs by recruiting ribosomes [16]. Competing endogenous RNAs (ceRNAs) are long noncoding RNAs that do not act directly on mRNAs. These ceRNAs trap miRNAs, thereby inhibiting the binding of miRNAs to their target mRNAs and increasing protein levels [17]. The *L-IST* has a complementary sequence to the SECIS sequence of *SELENOP* mRNA. *L-IST* appears to bind directly to *SELENOP* mRNA, inhibiting the binding of SBP2 to SECIS and of *SELENOP* mRNA to the ribosome. The mechanism of translational suppression of SELENOP by *L-IST* differs from the previously reported mechanisms of regulation of protein levels by long noncoding RNAs such as *antisense BASE-1*. However, Long-noncoding RNAs that bind directly to mRNAs, and inhibit the binding of SECIS to SECIS binding proteins such as SBP2 have not been reported. Therefore, the translational suppression of SELENOP by *L-IST* is considered to be a novel function of a noncoding RNA.

6. Effects of antisense RNA on selenoproteins other than SELENOP

We are currently analyzing the functional sequence of *L-IST* and its effect on the translation of other selenoproteins. We have not yet completely determined the precise functional sequences of *L-IST*. Nevertheless, we have identified the functional sequences that are present in both the 5′ and 3′ regions and not only sequences complementary to *SELENOP* mRNA [4]. Furthermore, it is becoming obvious that the complementary sequence between *L-IST* and *SELENOP* mRNA is not necessary for the suppression of *SELENOP* mRNA translation by *L-IST*. We replaced the complementary sequence region to *SELENOP* mRNA in *L-IST* with the sequence complementary to the 3′ UTR in other selenoprotein mRNA. The available data are preliminary, but they suggest that RNAs containing antisense sequences of 3′ UTR may also suppress protein levels of other selenoproteins as well as SELENOP. In the future, we will confirm the reproducibility of the study and analyze the effect of antisense long noncoding RNAs on the translation of mRNAs containing SECIS.

Conclusion

We identified a novel noncoding RNA, *CCDC152/L-IST*, with a sequence complementary to the SECIS sequence of *SELENOP* mRNA. *L-IST* reduced SELENOP protein levels by specifically inhibiting the translation step of *SELENOP* mRNA (**Figure**) [4]. SELENOP was originally studied as a Se transport protein, but recent studies have shown that excess SELENOP is associated with many diseases, such as T2DM [1,2]. As *L-IST* can specifically decrease SELENOP protein levels, therapies that increase *L-IST* may be promising for treating several diseases in patients with high SELENOP levels.

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References

[1] Mita Y, Nakayama K, Inari S, Nishito Y, Yoshioka Y, Sakai N, Sotani K, Nagamura T, Kuzuhara Y, Inagaki K, Iwasaki M, Misu H, Ikegawa M, Takamura T, Noguchi N, Saito Y. Selenoprotein P-neutralizing antibodies improve insulin secretion and glucose sensitivity in type 2 diabetes mouse models. *Nature Commun*. 2017; 8: 1658.

[2] Misu H, Takamura T, Takayama H, Hayashi H, Matsuzawa-Nagata N, Kurita S, Ishikura K, Ando H, Takeshita Y, Ota T, Sakurai M, Yamashita T, Mizukoshi E, Yamashita T, Honda M, Miyamoto K, Kubota T, Kubota N, Kadowaki T, Kim HJ, Lee IK, Minokoshi Y, Saito Y, Takahashi K, Yamada Y, Takakura N, Kaneko S. A liver-derived secretory protein, selenoprotein P, causes insulin resistance. *Cell Metab*. 2010; 12: 483-95.

[3] Bulteau AL, Chavatte L. Update on Selenoprotein Biosynthesis. (2015) *Antioxid Redox Signal*. 2015; 23: 775-94.

[4] Mita Y, Uchida R, Yasuhara S, Kishi K, Hoshi T, Matsuo Y, Yokooji T, Shirakawa Y, Toyama T, Urano Y, Inada T, Noguchi N, Saito Y. Identification of a novel endogenous long non-coding RNA that inhibits selenoprotein P translation. *Nucleic Acids Res*. 2021; 49: 6893-6907.

[5] Duntas LH, Benvenga S. Selenium: an element for life. *Endocrine*. 2015; 48: 756-75.

[6] M. P. Rayman, Selenium and human health, *Lancet*. 2012; 379: 1256–68.

[7] Vindry C, Ohlmann T, Chavatte L. Translation regulation of mammalian selenoproteins. *Biochim Biophys Acta. Gen. Subj*. 2018; S0304-4165, 30142-9.

[8] Low SC, Grundner-Culemann E, Harney JW, Berry MJ. SECIS-SBP2 interactions dictate selenocysteine incorporation efficiency and selenoprotein hierarchy. *EMBO J*. 2000; 19: 6882-90.

[9] Donovan J, Copeland PR. Selenocysteine insertion sequence binding protein 2L is implicated as a novel post-transcriptional regulator of selenoprotein expression. *Plos One*. 2012; 7: e35581.

[10] Romagné F, Santesmasses D, White L, Sarangi GK, Mariotti M, Hübler R, Weihmann A, Parra G, Gladyshev VN, Guigó R, Castellano S. SelenoDB 2.0: annotation of selenoprotein genes in animals and their genetic diversity in humans. *Nucleic Acids Res*. 2014; 42: D437-D443.

[11] Liu Z, Yan W, Liu S, Liu Z, Xu P, Fang W. Regulatory network and targeted interventions for CCDC family in tumor pathogenesis. *Cancer Lett*. 2023; 565: 216225.

[12] Huang J, Shi T, Ma T, Zhang Y, Ma X, Lu Y, Song Q, Liu W, Ma D, Qiu X. CCDC134, a novel secretory protein, inhibits activation of ERK and JNK, but not p38 MAPK. *Cell Mol Life Sci*. 2008; 65: 338-49.

[13] Kikuchi N, Satoh K, Kurosawa R, Yaoita N, Elias-Al-Mamun M, Siddique MAH, Omura J, Satoh T, Nogi M, Sunamura S, Miyata S, Saito Y, Hoshikawa Y, Okada Y, Shimokawa H. Selenoprotein P Promotes the Development of Pulmonary Arterial Hypertension. *Circulation*. 2018; 138: 600-623.

[14] Meister G, Landthaler M, Patkaniowska A, Dorsett Y, Teng G, Tuschl T. Human Argonaute2 Mediates RNA Cleavage Targeted by miRNAs and siRNAs. *Molecular Cell*. 2004; 15(2): 185-197.

[15] Faghihi MA, Modarresi F, Khalil A, Wood D, Sahagan B, Morgan T, Finch C, St Laurent G 3rd, Kenny P, Wahlestedt C. Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of beta-secretase. *Nature Med*. 2008; 14: 723-30.

[16] Carrieri C, Cimatti L, Biagioli M, Beugnet A, Zucchelli S, Fedele S, Pesce E, Ferrer I, Collavin L, Santoro C, Forrest A, Carninci P, Biffo S, Stupka E, Gustincich S. Long non-coding antisense RNA controls Uchl1 translation through an embedded SINEB2 repeat. *Nature*. 2012; 491: 454-7.

[17] Tay Y, Kats L, Salmena L, Weiss D, Tan S, Ala U, Karreth F, Poliseno L, Provero P, Cunto F, Lieberman J, Rigoutsos I, Pandolfi P. Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. *Cell*. 2011; 147: 344-57.