Alternative splicing of CLK1 in the THP1 cell line

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Abstract

Eukaryotes have the ability to alternatively splice RNA transcripts, which increases the number of proteins that a gene can code for. CLK1 play an important role in the regulation of alternative splicing. When THP1 cells were treated with the gymnema, exon4 of CLK1 was spliced out less often than in untreated cells. The exclusion of exon4 may cause the resulting protein to lose normal function. Cells treated with gymnema are probably more efficient in producing the complete CLK1.

Key words: CLK1, THP1, alternative splicing

Introduction

Alternative pre-mRNA splicing in higher eukaryotes enhances transcriptome complexity and proteome diversity. Its regulation is mediated by a complex RNA-protein network that is essential for the maintenance of cellular and tissue homeostasis. Disruptions to this regulatory network underlie a host of human diseases and contribute to cancer development and progression. The splicing kinases are an important family of pre-mRNA splicing regulators, which includes the CDC-like kinases (CLKs), the SRSF protein kinases (SRPKs) and pre-mRNA splicing 4 kinase (PRP4K/PRPF4B) [1,2,6].

These splicing kinases regulate pre-mRNA splicing via phosphorylation of spliceosomal components and serinearginine (SR) proteins, affecting both their nuclear localization within nuclear speckle domains as well as their nucleocytoplasmic shuttling [6].

CDC-like kinase 1 (CLK1) was identified as a splicing kinase in 1996 when a yeast 2 hybrid screen using Clk/sty (Clk1) kinase as bait identified 5 SR proteins as binding partners [4,5]. The authors went on to show that one of the interacting SR proteins, ASF/SF2 (SRSF1), was phosphorylated within its RS domain by Clk/sty, and that overexpression of Clk/sty, much like SRPK1, caused a redistribution of SR proteins from nuclear speckles, to a ubiquitous nucleoplasm localization [8,9].

The cdc2-like kinases (CLKs) are evolutionarily conserved kinases with dual specificity. The CLK family consists of four isoforms: CLK1, CLK2, CLK3 and CLK4. CLK1 play an important role in the regulation of alternative splicing of human genes through multisite phosphorylation of their serine/arginine-rich (SR) family of splicing factors [3,5,6].

In this experiment, the THP-1 cell line is derived from the suspension system. The RNA is extracted from the THP1 cell line based on the standard operation. CLK1 is universally exist in different cell lines, we choose THP1 cell line for our research in our lab; Gymnema can reduce cravings for sweets, sugar absorption during digestion. Someone in our lab has found the gymnema could induce the different expression of CLK1 in the THP1 cell line, so we choose the cell line treated with gymnema. The purpose of this study was to determine the effects of the nutraceutical gymnema on the alternative splicing of the CLK1 transcript in THP1 cells.

Material and method

Cell line

The THP1 cell lines are provided by Prof. Rubin's laboratory; untreated cell line and cell line treated with Gymnema;

RNA extraction

RNA was extracted from untreated cell line and cell line treated by Gymnema; using RNeasy® Plus Mini Kit (QIAGEN), according to the manufacturer's instructions.

Primers

One pair of primers was designed for this experiment (Table 1). The expected size of product is about 516 bp. The purified RNAs were amplified with RT-PCR using primers in exon 2 and 5 of CLK1 (Figure 1).

RT-PCR

RT-PCR was performed using QIAGEN® One-Step RT－PCR Kit following instructions. Ten nanograms of RNA was amplified in 10 μl RT-PCRs (2 μl 5×RT buffer, 0.4 μl 10 mM dNTPs, 0.5 μl 10 pmol/ μl forward primer, 0.5 μl 10 pmol/ μl reverse primer, 0.4 μl enzyme mix, 2 μl 5 ng/ μl RNA and 4.2 μl ddH2O).

Temperature cycles as follow: one cycle of 50 ℃ for 30min and 95 ℃ for 15min, 94 ℃ for 30 s, 58 ℃ for 30s, and 72℃ for 30s, and a final extension of 72 ℃for 5 min. followed by a final hold at 4℃. Cycle number was 50.

Electrophoresis

2 μl of loading dye was added to each RT-PCR product. 5 μl of each product was then added to a 1% agarose gel, and electrophoresis was performed at 160 V. Band intensities were visualized by ethidium bromide in a UV trans-illuminator (BioRad). 7 μl of 100 bp marker was used to measure the size of bands.

Gel extraction and sequencing

The target products was extracted by QIAquick Gel Extraction Kit(QIAGEN) following the manufacturer's instructions and subsequently sequenced by GENEWIZ® in order to identify PCR products.

	Sequence (5'->3')	Length	Tm	GC%	Expected size of products
Forward primer 2F	GTCCTGATTGGGATGACAAG	20	55.81	50	516 bp
Reverse primer 5R	GATCGATGCACTCCACAAC	19	56.43	52.63	

Tabel 1. The summary of primers and the size of expected products

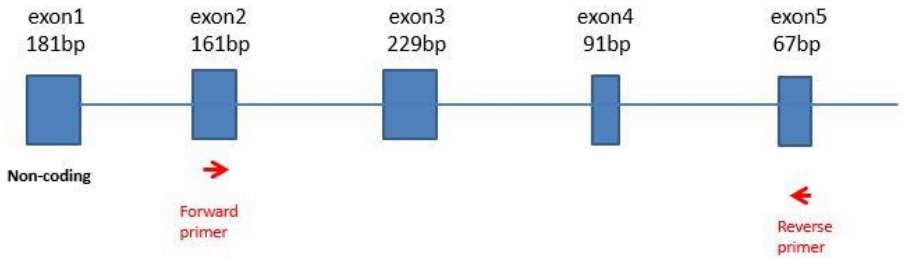

Figure 1. Location of RT-PCR primers in exons2 and 5 in CLK1

Results

Spliced variants of CLK1 were observed and reading frameshift

The primers in exons2 and 5 amplified two products in both treated and untreated cells. However, the relative quantities of two bands differed between treated and untreated cells (Figure 2). Sequencing confirmed that variant A contains exon 4, while variant B lacks this 91bp exon (Figure 3). Variant A codes for the full-length, functional CLK1 protein, which is 484 amino acids in length. The exclusion of exon 4 causes a frameshift mutation, leading to an early stop codon in variant B and a protein product 136 amino acids in length.

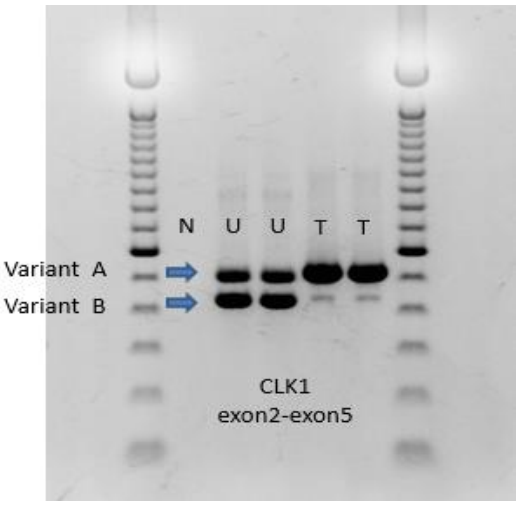

Figure 2. Agarose gel electrophoresis of products from RT-PCR using primers in exons 2 and 5 of CLK1.

U = RNA extracted from untreated THP1 cell

T = RNA extracted from THP1 cell treated with gymnema

N =non-template control

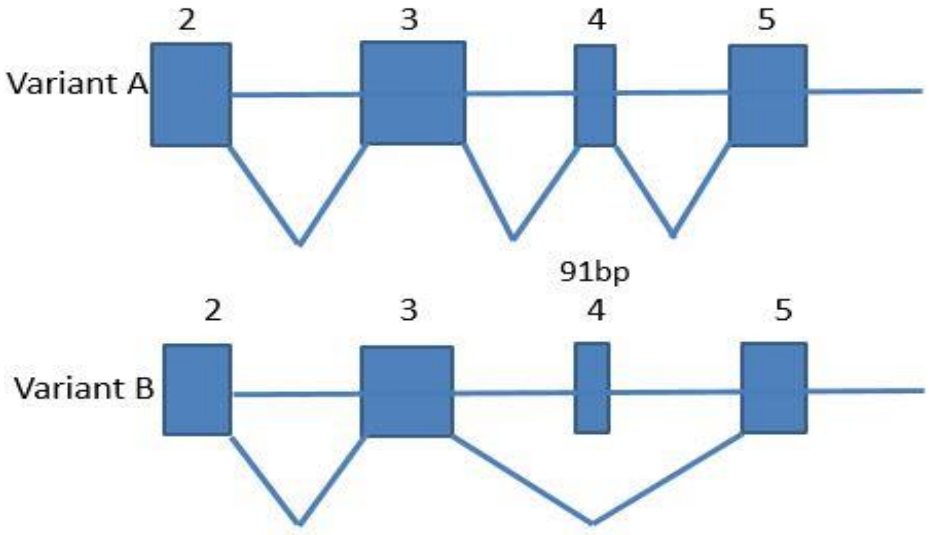

Figure 3. Alternatively spliced transcripts of CLK1 observed in this study. Variant A codes for a functional CLK1 protein. The exclusion of exon4 in variant B causes a frameshift mutation and an early stop codon.

The relative proportions of variants A and B differed between treated and untreated cells. In untreated cells, variant A for CLK1 (form containing exon 4) was approximately 44% of the CLK1 transcripts amplified by the RT-PCR. In cells treated with gymnema, variant A made up approximately 92% of the transcripts amplified.

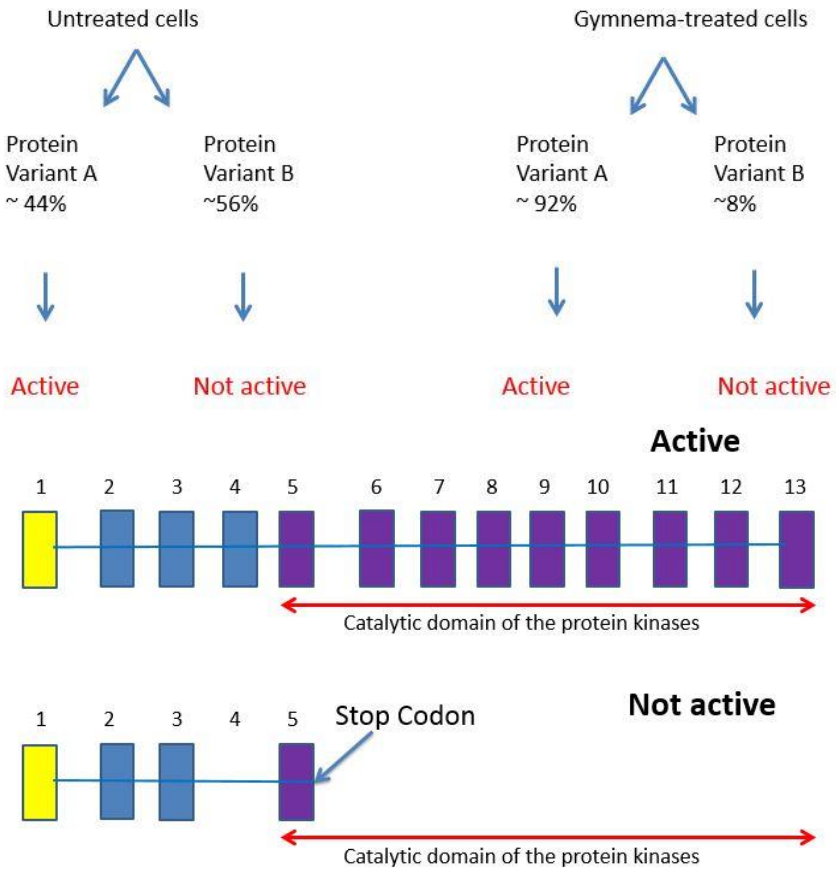

Figure 4. Results of Image J quantification of the two transcripts observed in this study and a diagram of the potential impacts of containing or lacking exon 4 on the protein levels.

Discussion

Because of the role of CLK1 in alternative splicing of transcripts of other genes, changing splicing of CLK1 in cells could have a major impact on the proteins synthesized and, thereby, cellular function. In this study, treatment with gymnema caused THP1 cells to produce relatively more CLK1 transcript containing exon 4 (variant A) and less transcript lacking exon4 (variant B) compared to untreated cells. Splicing out exon 4 causes a frameshift mutation that creates an early stop codon, resulting in a truncated protein. The catalytic domain of CLK1 is encoded by

exon 5 through 13 (Figure 4). As a result, exclusion of exon 4 will very likely alter the function of the resulting protein.

Gymnema, a nutraceutical extracted from the leaves of the herb Gymnema sylvestre (Figure 5), may cause cells to produce more functional CLK1 protein. These results add to a growing body of research indicating that nutraceuticals may be used to treat a variety of ailments by changing how various transcripts are spliced.

Figure 5. Leaves of the plant Gymnema sylvestre

Conclusion

From the result of experiment, we could get the following conclusion: Protein Variant A is larger than Protein Variant B. Protein variant A has function; protein variant B has no function. Most of the protein in treated cell line is protein variant A. The treated cell line would be more efficient in producing the complete CLK1.

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