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Original article

Resveratrol enhances splicing of insulin receptor exon 11 in myotonic dystrophy type 1 fibroblasts

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Abstract

Introduction: Myotonic dystrophy type 1 (DM1) is characterized by splicing abnormalities caused by CUG expansion of the *DMPK* gene transcript. Splicing of exon 11 of the insulin receptor (*IR*) gene is deregulated to suppress exon 11 inclusion into mRNA in DM1. Consequently, the exon 11-deleted IR isoform that is less sensitive to insulin is predominantly produced, leading to glucose intolerance in DM1. Upregulation of exon 11 retaining full-length IR mRNA is a potential way to recover insulin sensitivity in DM1.

Methods: We examined candidate chemicals for their ability to enhance inclusion of exon 11 of the *IR* gene in cultured cells by reverse transcription-PCR amplification of a fragment extending from exons 10 to 12 of *IR* mRNA.

Results: We revealed that resveratrol (RES) enhanced the percentage of exon 11-containing *IR* mRNA among the total *IR* mRNA in HeLa cells. The RES-mediated enhancement of exon 11 inclusion was cell-specific and highest in fibroblasts. We tested RES on four fibroblast samples from three generations of one DM1 family. In each sample, RES treatment significantly upregulated the percentage of exon 11-containing IR mRNA to levels higher than that of the control, irrespective of the length of the sample's CTG repeat expansion.

Discussion: A natural compound, RES, was shown for the first time to upregulate the full-length IR mRNA in fibroblasts from DM1 cases. Our results provide the justification of RES as a leading compound to improve glucose tolerance in DM1.

Keywords: Resveratrol; Insulin receptor; Splicing; Myotonic dystrophy 1

1. Introduction

Myotonic dystrophy type 1 (DM1) is the most common type of muscular dystrophy in adults and is characterized by myotonia, progressive myopathy, and multiorgan involvement including glucose intolerance [1]. DM1 is an autosomal dominant disorder with a tendency to worsen in successive generations (anticipation) and a maternal transmission bias for congenital forms [1]. DM1 is caused by expansion of a CTG repeat in the 3′-untranslated region of the dystrophia myotonica protein kinase (*DMPK*) gene on chromosome 19. The DM1 mutation shows a propensity to expand through

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successive generations, providing a molecular basis for genetic anticipation. Repeat lengths of 38–50 and 51–100 are considered premutation and protomutation, respectively, and both show instability towards expansion. Patients with the adult-onset form carry more than 100 repeats, and those with the congenital form have more than 1000 repeats [2].

DM1 can be considered a spliceopathy [3], because the transcribed CUG repeats bind the RNA splicing regulator muscleblind-like 1 protein (MBNL1), causing splicing deregulation in tens to hundreds of genes [4].

The insulin receptor (*IR*) gene is one of the genes whose splicing is deregulated in DM1 [5]. The *IR* gene consists of 22 exons and exon 11 is alternatively spliced to produce two isoforms: insulin receptor A (IRA) that lacks the exon 11 sequence, and insulin receptor B (IRB) that retains exon 11. IRA has a lower insulin sensitivity than does IRB. In DM1, the predominant production of IRA causes glucose intolerance, one of the most common complications of DM1 [5,6].

Splicing modulation to enhance full-length IR mRNA production is potentially a very useful way to improve glucose tolerance in DM1. Many studies on splicing modulation of exon 11 of the *IR* gene have been conducted [6,7]. Pentamidine, an antimicrobial medication, has been shown to reverse the splicing defects associated with DM1 using an *in vitro* splicing system [8]. However, there remain questions before its clinical application for DM1 treatment, because of its immune-depressing side effect.

Resveratrol (RES) is a polyphenolic flavonoid found in grape skins and seeds, red wine, blueberries, mulberries, peanuts, and rhubarb [9]. Beneficial effects of RES on many biological processes have been reported. Recently RES has been reported to modulate splicing to produce favoring mRNA in mutation-induced splicing errors [10,11]. Here, we explored modulation of splicing of exon 11 of the *IR* gene using HeLa cells. We found that RES, a natural compound, enhanced inclusion of exon 11 of the *IR* gene. Remarkably, RES enhanced exon 11 splicing in all four fibroblast lines obtained from three generations of a DM1 family. We believe RES to be a highly promising compound to improve glucose tolerance in DM1.

2. Materials and methods

2.1. Chemicals

To test the effect of chemicals on *IR* exon 11 splicing, various chemicals were dissolved in the culture medium. Candidate chemicals (kinetin, kinetin riboside, TG003, SRPIN340, RES, epigallocatechin gallate, 3-nitropropionic acid, and prednisolone were chosen based on their published splicing modulation activity [12–16]. SRT1720 (Calbiochem, San Diego, CA) and 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) (Wako Pure Chemical Industries, Kyoto, Japan) were used at 0.1 μM and 1 mM, respectively. AICAR was dissolved in water. The other chemicals were dissolved in 0.1% dimethyl sulfoxide (DMSO) (Wako Pure Chemical Industries).

2.2. Cell culture

The cervical epithelial adenocarcinoma cell line (HeLa), the human metastatic neuroblastoma cell line (SH-SY5Y), the hepatocarcinoma cell line (HepG2), and the embryonic kidney cell line (HEK293) cells were obtained from ATCC (Manassas, VA). Skeletal muscle cells were obtained from Lonza (Walkersville, MD). Fibroblasts from control and DM1 patients were obtained from the Coriell Institute for Medical Research (Camden, NJ).

HeLa cells and other cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with 5% and 10% fetal bovine serum (Invitrogen, Carlsbad, CA), respectively. HeLa cells were incubated in the presence of chemical compounds for 8 h in the screening test. HeLa cell and other cells were incubated for 24 h and harvested for further examination.

2.3. Analysis of splicing of IR exon 11

RNA was isolated from cultured cells using High Pure RNA Isolation Kit (Roche Applied Science, Mannheim, Germany) and reverse-transcribed using the random hexamer primers with M-MLV Reverse Transcriptase (Invitrogen-Life Technologies, Carlsbad, CA) as described previously [13]. A fragment of *IR* mRNA extending from exon 10 to exon 12 was PCR amplified using a set of primers (*IR*: F 5′-CCAAAGA CAGACTCTCAGAT-3′ and R 5′-AACATCGCCAA GGGACCTGC-3′); the human glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA was also PCR amplified as described before [13]. The PCR conditions were as follows; initial denaturation at 94 °C for 3 min followed by 28 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 90 s, and a final extension at 72 °C for 3 min. Reverse transcription (RT)-PCR products were separated and semi-quantitated by measuring the peak areas of high-resolution capillary electrophoresis using an Agilent 2100 Bioanalyzer with a DNA1000 LabChip kit (Agilent Technologies Inc., Santa Clara, CA). PCR size determination and quantification were automatically performed using 2100 Expert software (Agilent Technologies Inc.). The sequences of all the detected bands were confirmed by subcloning and sequencing as previously described [13]. All samples were run in triplicate. The percentages of exon 11-containing *IR* mRNA (exon 11 + IR) were calculated using the