Original Article
LncRNA UCA1 inhibits cell proliferation in coronary heart disease

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Abstract: According to the length of nucleotides, non-coding RNA is normally divided into two types. The length of nucleotides that < 200 nt can be called small RNA. In contrary to this, the length of nucleotides > 200 nt can be named long RNA. Many studies have shown that a large number of lncRNAs could play important roles in biological mechanisms for many types of coronary heart diseases, such as proliferation and apoptosis. In this study, the lncRNA UCA1 as a new lncRNA was examined in coronary heart disease. In order to examine the biological roles of lnrRNA UCA1 in coronary heart disease, RT-PCR and MTT were utilized in this research. The experimental results showed that overexpression of UCA1 could inhibit the proliferation of VSMC cells. Moreover, overexpression of UCA1 can inhibit the expression of OPN.

Keywords: lncRNA UCA1, RT-PCR, MTT, proliferation, coronary heart disease

Introduction

Coronary heart disease is a major type of heart diseases, which seriously damaged the health of people all over the world. The coronary heart disease was the seventh death cause for people in our country in 1987. However, it already turned into the second cause in our nation since 2010 [1]. The standardized administration of antiplatelet drugs has been utilized to reduce the risk of further coronary heart disease-associated events [2, 3]. Although there have been many signs of progress in the treatment of coronary heart disease, the mortality remains high. The development of coronary heart disease is through a complicated procedure, which has relative to many genetic variations [4-6]. Most importantly, the proliferation of endothelial cells is the main cause of the coronary heart disease [7, 8]. Therefore, it is urgent for us to find the biological role of UCA1 in the proliferation of endothelial cells.

There have been many signs of progress in the next sequencing technologies in the past few years, many studies have shown that only 10% of genes which were transcribed into coding RNAs, but 90% of the RNAs can be named the non-coding RNAs [5, 6, 9]. The non-coding

RNAs (ncRNAs) could be divided into two types that could be based on the size, the length of small ncRNAs are < 200 nt and the length of long ncRNAs are > 200 nt [10]. There are more and more reports have shown that miRNAs can be regarded as oncogenes or genes that could inhibit the tumor [11-13]. In the contrast, lncRNAs were regarded as the transcriptional ones. Moreover, the lncRNAs could play an important role in many biological progress, including X chromosome inactivation, chromatin remodeling, and transcriptional repression [14-16].

In this study, we find a new lncRNA, named lncRNA UCA1. This lncRNA was located on the chromosome 12. The results have shown that overexpression of UCA1 could inhibit the proliferation of endothelial cells. Moreover, overexpression of UCA1 could decrease the expression of OPN.
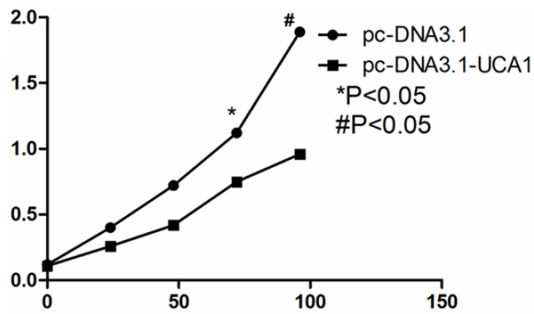
Materials and methods

Cell culture

The VSMC cells were purchased from shanghai hongshun cell bank. The cell lines were cultured in RPMI-1640 medium (Sigma, USA). The medi-
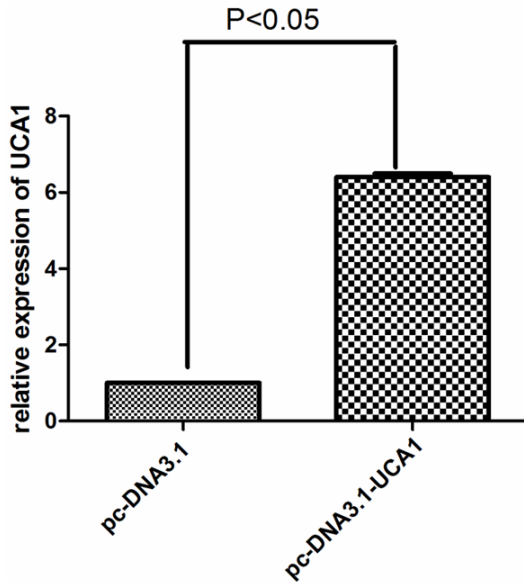
Figure 1. Relative expression levels of UCA1 in the pc-DNA3.1 group and pc-DNA3.1-UCA1 group (p < 0.05).

Figure 2. MTT assays were performed after transfection to determine the cell viability of the transfected VSMC cells (p < 0.05).

um was replenished with 10% fetal bovine serum (FBS), two antibiotics namely penicillin and streptomycin (all from Sigma, USA) at 37°C in a 5% CO_2-95% air atmosphere.

Cell transfection

VSMC Cells were transfected with 75 nM of pc-DNA3.1-UCA1 expression plasmid and pc-DNA3.1 vector, (all purchased from Genema, Changsha, China). The pcDNA 3.1 transfected by using lipo 3000 (Thermo Fisher Scientific, Inc.).

RT-PCR for UCA1

The total RNA from VSMC cell lines and the total RNA was extracted and treated with DNase.
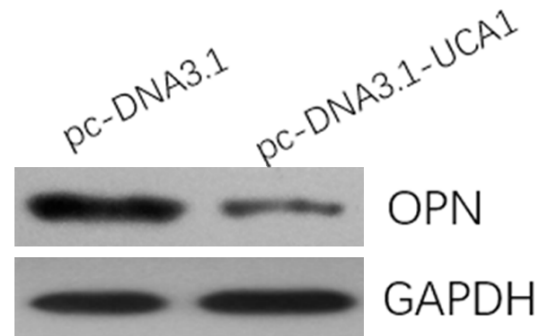
**Figure 3.** The relative protein level of OPN after overexpression of UCA1.

The quantitative RT-PCR analysis was performed using an ABI Prism 7300 sequence detector (Applied Biosystems). PCR primers for UCA1 are CAGTGTGAATTACAGCAAACC (forward) and ACAGTGTATCAGTGAAGGAAT (reverse). The primers for OPN are TAGGGTCGAGGCTATCGGA (forward), GGATCGCGGCTATTATAGC (reverse). The reaction was performed in 25 μl with 1.5 μl of cDNA, 2 × Platinum SYBR Green qPCR SuperMix UDG (Invitrogen), and 300 nM primers. PCR conditions were two minutes at 50°C, two minutes at 95°C, and 40 cycles of 15 seconds at 95°C and 45 seconds at 60°C. The target and reference genes were amplified in separate wells. All reactions were performed in duplicate. Reaction mixture without the cDNA was used as a negative control in each trial. CT values normalized to 18 s were used to compare the difference.

MTT assay

VSMC cells were suspended and planted into 96-well plates (5 × 10^4 cells/well) and cultured for 0, 24, 48, 72 and 96 h using RPMI-1640 medium with 10% FBS at 37°C. The viability of VSMC cells was examined using an MTT assay. Briefly, following cell culture, add 20 μl CCK (Sigma, USA) to each well and incubated at 37°C for 4 h. The resulting formazan product was dissolved in 100 μl isopropanol and the absorbance was at 490 nm.

Statistical analysis

All statistical analyses were performed using SPSS 22.0 software (IBM, SPSS, USA). The significant differences between groups were estimated by Student's t-test and the X^2 test as appropriate. Two-sided *P* values were calculated, and a probability level of 0.05 was considered to be statistically significant.
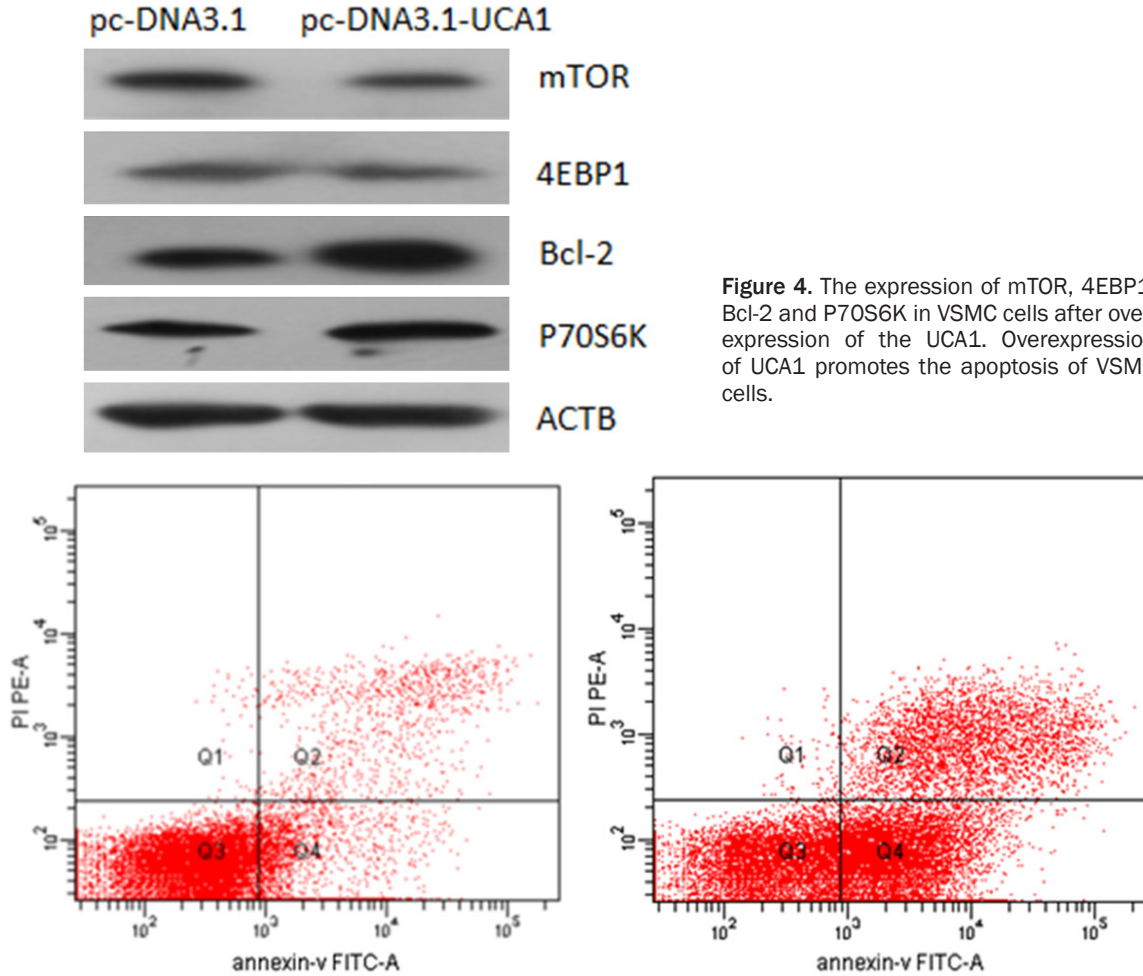
Figure 4. The expression of mTOR, 4EBP1, Bcl-2 and P70S6K in VSMC cells after overexpression of the UCA1. Overexpression of UCA1 promotes the apoptosis of VSMC cells.

Results

In order to detect the biological role of lncRNA UCA1 in VSMC cells line, the pc-DNA3.1-ATB was synthesized and transfected into the VSMC cell lines. The RT-PCR was utilized in this study. The experimental results showed that the expression of UCA1 in experimental group pc-DNA3.1-UCA1 was increased by 300% (**Figure 1**) compared to the pc-DNA3.1 experimental group. After that, MTT was used to detect the proliferation of cells after transfected the pc-DNA3.1 into VSMC cell lines. The experimental results showed that overexpression of UCA1 could inhibits the proliferation of VSMC cells (**Figure 2**). Moreover, the experimental results also showed that overexpression of UCA1 could inhibits the expression of OPN (**Figure 3**). The expression of mTOR, 4EBP1, Bcl-2, and P70S6K in VSMC cells after overexpression of the UCA1 were also characterized (**Figure 4**). Finally, the experimental

results from the flow cytometry showed that overexpression of UCA1 could promote the apoptosis of VSMC cells (**Figure 4**).

Discussion

The cell proliferation could be one of the causes of coronary heart disease. Moreover, overexpression of UCA1 can inhibit the expression of OPN, which is related to the coronary heart disease. The mammalian target of rapamycin (mTOR), a major gatekeeper of anabolism, is a protein serine/threonine kinase that could give rise to two complexes with different sensitivity to rapamycin [17, 18]. A main functional role of mTORC1 is the regulation of mRNA translation via its targets 4EBPs and S6Ks. Unphosphorylated 4EBP1 binds to the cap-binding complex and sequesters eIF4E away from it, which could result in a block of cap-dependent mRNA translation [19, 20]. Therefore, the mTOR signal pathway plays an important role in occurrence

and development of cancer including OS, which is also one of the focus of this study.

In conclusion, overexpression of UCA1 can influence the cell proliferation, which has a compact relationship with coronary heart disease. Moreover, overexpression of UCA1 could influence the expression of OPN, which also related to coronary heart disease.

Disclosure of conflict of interest

None.

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