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RELATIONSHIP BETWEEN OXIDIZED LOW-DENSITY LIPOPROTEIN TREATMENT AND HEPATOCELLULAR CARCINOMA ASSOCIATED TRANSCRIPT 1 EXPRESSION

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Abstract: Long non-coding RNAs (lncRNAs) have multiple biological functions in the vascular system including in endothelial cells (ECs). Hepatocellular carcinoma associated transcript 1 (HULC) is an lncRNA and highly up-regulated in liver cancer. Oxidized low-density lipoprotein (ox-LDL) is a remarkable risk factor for various disease. We investigated and compared HULC expression in human umbilical vein endothelial cells (HUVECs) treated with two different concentration of ox-LDL. We used quantitative polymerase chain reaction (qPCR) method to detect HULC expression level in HUVECs. HULC expression was found to be statistic significantly up-regulated in HUVECs treated with higher concentration of ox-LDL ($P<0.001$). It may be put forward that high concentration of ox-LDL treatment may be inducer of HULC gene expression in variety of diseases including in cancer and cardiovascular diseases (CVDs).

Keywords: Hepatocellular carcinoma associated transcript 1, Oxidized low-density lipoprotein, Gene expression, Quantitative polymerase chain reaction

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1. Introduction

Genome sequences (70-90%) of humans are transcribed into RNAs. Little of them are encoded into proteins through translation. The most part of these stay at RNA form that do not code protein and they performed functional regulators in biological process (Chen et al., 2020). Non-coding RNA molecules (ncRNAs) compose of RNA groups without protein-coding function. NcRNAs plays role in various levels of gene expression such as epigenetic, transcription and, post-transcriptional regulation (Chen et al., 2020). NcRNAs can be classified according to nucleotides (nt) size. LncRNA group is ncRNA subcategorie which have greater than 200 nt (Meng et al., 2020). LncRNAs are known to regulate pathological mechanisms and progression of diseases via different target genes or signaling pathways, thus they can be accepted essential biomarkers (Ghafouri-Fard et al., 2021). *HULC* is the first lncRNA that is proved to be strongly overexpressed in human hepatocellular carcinoma (Abbastabar et al., 2018). *HULC* gene is located on chromosome 6. A spliced, polyadenylated lncRNA of approximately 500 nt in length, which is localized in the cytoplasm is generated by the transcription of *HULC* gene. It has been reported that *HULC* is involved in function of ribosome (Chen et al., 2020). Endothelial cells (ECs) are inner layer of blood vessels. The ECs are accepted important component of vascular homeostasis (Lifeline Cell Technology, 2020). Ox-LDL is one of the crucial factors causing to ECs activation, dysfunction, and injury. The multifactorial role of ox-LDL in blood vessels makes it a prime candidate for exploring new disease mechanisms responsible for endothelial dysfunction and CVDs. It is an ideal target for developing new cardiovascular drugs (Jiang et al., 2022). Furthermore, epidemiological studies have shown that ox-LDL are closely linked with colorectal cancer, breast cancer, pancreatic cancer, and other malignancies, suggesting that it plays critical roles throughout the cancers occurrence and development (Deng et al., 2022). ECs are critical players which effect development of CVDs and a variety of cancers. EC lines are important to research vascular disease and cancer (Lifeline Cell Technology, 2020). Thus, we used HUVECs that are EC line isolated from the umbilical blood vessel in this study. LncRNAs may change transcriptional events related with impairment of the ECs (Weirick et al., 2018). Endothelial dysfunction is one of the main causes for development of pathological disorders and is highly associated with the lncRNAs dysregulation (Jayasuriya et al., 2022). In addition, ox-LDL treatment may alter lncRNAs expression in ECs to effect endothelial dysfunction (Jiang et al., 2022). Based on all these knowledge, our study hypothesis was that the gene expression of *HULC*, a lncRNA, may change after increasing ox-LDL treatment in HUVECs. Using the HUVEC line in this study is valuable in confirming our hypothesis.

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2. Materials and Methods

2.1. Cell Line

We used HUVECs as cell line materials in all experiments. HUVEC line provided from American Type Culture Collection (ATCC). HUVECs were cultured according to the datasheet from ATCC. HUVECs were cultured in Dulbecco's modified Eagle's medium (DMEM, High glucose (4.5 g/l) with L-Glutamine; CAPRICORN) with fetal bovine serum (FBS, Advanced collected in South America; CAPRICORN) and penicillin/streptomycin solution (100x; CAPRICORN) at 37 °C in an incubator with 5% CO_2. HUVECs were added with 25 and 40 µg/ml ox-LDL and then incubated in 24 hours at 37 °C in an incubator with 5% CO_2. Cell density decreased significantly after the application of 40 µg/ml ox-LDL in HUVECs. Therefore, we determined our experimental group as HUVECs treated with 40 µg/ml ox-LDL. Ox-LDL concentration level at which cell viability began to be affected was 25 µg/ml (Ma et al., 2024), and experiments were carried out with these two ox-LDL concentrations.

2.2. RNA Isolation and qPCR

In RNA isolation step, total RNA was isolated from HUVECs through RNeasy Mini Kit (QIAGEN, catalog no:74104). We performed complementary DNA (cDNA) synthesis by utilizing reverse transcription kit (A.B.T.™ with RNase Inh. High Capacity, catalog no:C03-01-20). At last, *HULC* gene expression was detected QPCR using cDNA, RNase free water (nzytech, MB11101), SYBR Green (A.B.T.™ 2X qPCR SYBR-Green MasterMix kit, catalog no:Q03-02-01 ve Q03-02-05), and lncRNA qPCR primer of *HULC* (QIAGEN, catalog no:LPH17802A). *Glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) was used as internal control for qPCR experiments.

2.3. Statistical Analysis

"GeneGlobe Data Analysis Center" (https://geneglobe.qiagen.com/us/analyze QIAGEN, Hilden, Germany) was used to analyze expression data of HULC gene. We uploaded QPCR data to the analysis system. Threshold cycle (Ct or Cq) values which had been obtained from qPCR experiments were saved. Fold change (FC) value was calculated by ΔΔCt method after comparison of groups according to the qPCR data. FC and fold regulation (FR) were calculated as the ratio of the relative gene expression between the groups of HUVECs treated 25 µg/ml ox-LDL and group of HUVECs were treated 40 µg/ml ox-LDL. Numbers higher than 1 demonstrate up-regulation, numbers between 0 and 1 demonstrate down-regulation, and a FC value of 1 demonstrates no change (Livak and Schmittgen, 2001). P-value was calculated based on a Student's t-test of the replicate $2^{-\Delta Ct}$ values for each gene in groups' comparison (RT2 Profiler PCR Arrays & Assays Data Analysis Handbook, 2019). P-value is less than 0.05 was accepted significant.

3. Results

We divided our study population to 2 groups: HUVECs treated with 25 µg/ml ox-LDL and HUVECs treated with 40 µg/ml ox-LDL. Findings belonged to *HULC* gene expression result from qPCR was detected in groups. *HULC* gene expression was compared between HUVECs treated with 25 µg/ml ox-LDL and HUVECs treated with 40 µg/ml ox-LDL. We found that *HULC* gene expression was up-regulated 2.48 fold in HUVECs treated with 40 µg/ml ox-LDL compared to HUVECs treated with 25 µg/ml ox-LDL (Table 1). This increase was statistic significant ($P<0.001$). Table 1 shows mean of Ct values obtained from *HULC* and *GAPDH* gene expression experiment in both groups as well FC, FR results associated with *HULC* gene expression as a result of groups' comparison (Table1).

4. Discussion

Some ncRNAs exhibits tissue-specific expression, and thus they play a major role in cells. LncRNAs are known as novel ncRNA transcripts involved in epigenetic regulation of pathophysiology of the diseases. Advancement in genomic and transcriptomics have shown that ECs control normal physiological and pathological condition. This situation has supported with the arising of lncRNAs which regulate gene expression involved in endothelial development (Jayasuriya et al., 2022). Abnormal expression of them may cause epigenetic dysregulation (Esteller, 2011). Therefore, this situation contributes to development of the diseases. The lncRNAs have been found as molecular targets in many diseases, including CVDs and cancer (Hobuss et al., 2019; Jiang et al., 2019). Recently, researchers have found that many lncRNAs are abnormally expressed in biological samples obtained from patients with CVDs (Wu et al., 2023).

Table 1. Fold change of *HULC* expression in HUVECs after different concentration of ox-LDL treatment.

Gene	HUVECs treated with 25 µg/ml ox-LDL	HUVECs treated with 40 µg/ml ox-LDL	FC	FR	P value
HULC Mean of Ct	21.52	19.06	2.48	up-regulation	<0.001*
GAPDH Mean of Ct	28.54	27.39	1.00		Nan

HUVECs= human umbilical vein endothelial cells, ox-LDL= oxidized low-density lipoprotein, FC= fold change, FR= fold regulation, Ct/Cq= threshold cycle, HUVECs+ox-LDL= HUVECs treated with ox-LDL, *GAPDH* (control gene)= *Glyceraldehyde 3-phosphate dehydrogenase, *= P value<0.001.*

It was found that silencing of *HULC* can significantly decrease viability and migration of human mammary epithelial cells (Yin et al., 2018). *HULC* expression levels was found to down-regulated in heart tissues after myocardial infartion (P<0.05) and its expression was also decreased in cardiac microvascular ECs induced by hypoxia (P<0.05) according to the qPCR results. In the same study, it was implicated that *HULC* overexpression can repress expression of inflammatuar molecules to protect HUVECs from inflammation damages and promote angiogenesis to increase viability and proliferation of HUVECs induced by hypoxia (Chen et al., 2020). Ox-LDL is implicated that it increases oxidative stress and inflammation and abnormally regulates proliferation and migration in cells (Wu et al., 2021). In this study, we treated HUVECs with two different concentrations of ox-LDL (25 and 40 µg/ml) to detect whether *HULC* expression alteration in HUVECs. We found that *HULC* expression was up-regulated in HUVECS treated with higher concentration of ox-LDL (p<0.001) (Table 1). Increasing ox-LDL concentration in HUVECs may reverse the effects of ox-LDL by up-regulating *HULC* expression. To prove this, experiments such as apoptosis and proliferation after gene silencing in cell culture are necessary. *HULC* can be not only the diagnostic biomarkers but also potential therapeutic target according to results of CVD studies (Xie et al., 2022). Furthermore, numerous studies have revealed that many lncRNAs were differentially expressed in the transcriptome of cancer cells (Jin and Fan, 2024). *HULC* is often known as cancer-associated lncRNA (Jayasuriya et al., 2022). It has role in angiogenesis one of the common features of cancer (Hernández-Romero et al., 2019). The pro-angiogenic role of *HULC* in ECs has been shown (Yin et al., 2018). Elevated *HULC* expression in cells may activate proliferation of the cell, growth of tumor, and cause to tumor suppressor down-regulation (Du et al., 2012). A few studies have shown up-regulation of *HULC* in human cancers (Ghafouri-Fard et al., 2020). We found up-regulated *HULC* expression in HUVECs after a higher ox-LDL treatment in our current study too (Table 1). Consistent with these results, increasing in this gene expression may influence risk of cancer. Thus, *HULC* may be accepted as risk locus a few cancers in different population. Studies have demonstrated that *HULC* may be regarded as an oncogenic lncRNA and a potential diagnostic and prognostic biomarker for malignancy according to results from experiments in cell line, animal and human studies (Ghafouri-Fard et al., 2020).

5. Conclusion

Since *HULC* expression increases in HUVECs induced with higher concentrations of ox-LDL and *HULC* is known to be an oncogenic lncRNA, this situation may be an advantage for cancer cells. Silencing *HULC* may be useful for cancer treatment. On the contrary, since *HULC* has been shown to increase angiogenesis, proliferation and migration in previous heart studies, the up-regulation of *HULC* expression after higher concentration of ox-LDL treatment may be investigated in the treatment of CVDs. *HULC* may be considered an important potential lncRNA drug candidate for CVDs. Further molecular studies are needed to determine all of these.

Author Contributions

The percentages of the author contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	B.B.
C	100
D	100
S	100
L	100
W	100
CR	100
SR	100
PM	100

C=Concept, D= design, S= supervision, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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