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L1Hs and MPP8 genes expression correlation with several types of carcinoma tumors

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ABSTRACT

L1Hs is a major player in genome evolution and genetic variability. Aberrant activity of L1Hs element is common in several human cancers. It's specific function is poorly characterized epigenetic regulation via human silencing Hub (HUSH) complex is critical to inhibit L1Hs activity. MPP8 is a subunit of HUSH complex and recently it has been suggested to inhibit L1Hs elements transcription. Thus, this assay was aimed to assess the expression level of L1Hs and MPP8 genes in cancer whole blood samples and healthy group utilizing qPCR assay. Generally, L1Hs gene significantly up regulated two times in patients' blood samples compared with healthy group. MPP8 gene up regulated approximately two times in cancer samples compared with healthy group. Testes, prostate, colon and cervical cancers were significantly expressed L1Hs more than MPP8 expression. While there is no significant differ in expression level of MPP8 and L1Hs genes in breast, bladder and ovary cancers.

KEYWORDS: L1Hs, MPP8, carcinoma tumors

ARTICLE DETAILS

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INSTRUCTION

Approximately 50% of human genomic sequence is composed of transposons, also called transponslements (TE) or junk DNA. The only transpon can mobilize autonomously is L1 element that occupies approximately 17% (~500-1000) copies of total genomic DNA. Approximately 100 copies of L1Hs elements (full length) are able to transpose (Burns, 2017; Huang *et al*., 2012; Kazazian Jr and Moran, 2017). L1Hs encode two proteins (ORF1 and ORF2) proteins (Huang *et al*., 2012; Lander *et al.*; Payer and Burns, 2019). According to the auto mobilization activity of L1Hs can be considered almost element deleterious leading to chromosomal rearrangements, genomic instability and insertion mutagenesis, which contribute to cause dis orders of human DNA including cancer (Beck *et al*., 2011; Hancks and Kazazian, 2016; Payer and Burns, 2019). Changing transposed elements expression including L1Hs are companied with diseases including neural diseases, autoimmune disorders and cancers (Kazazian Jr and Moran, 2017; Rodriguez-Martin *et al.*, 2020; Thomas *et al*., 2017). Although, high abundance of L1Hs in genomic DNA sequence, L1Hs activity is naturally controlled via several mechanisms to cause genomic stability including DNA methylation (Deniz *et al.*, 2019; Jönsson *et al*., 2019). siRNA and chromatin modification (Chen *et al.*, 2012; Puszyk *et al*., 2013). H3k9me3 consider as the most chromatin modification to silence L1Hs expression in human DNA genome (Deniz *et al*., 2019; Healton *et al*., 2020). Human silencing hub (HUSHcomplex) is implicated in silencing genome of transposable elements. Recently this complex has been identified as repressing factor of L1Hsexpression via targeting full length of intronic L1Hs by deposition H3K9me3 on the L1Hs elements (L1) (Gu *et al*., 2021; Liu *et al.*, 2018). HUSH complex including three critical components, PPHLN1, TASOR and MPP8 and recruits SETDB1 and MORC2 effectors to deposition h3K9ME3 and chromatin compaction to silence L1Hs expression (Deniz *et al.*, 2019; Gu *et al.*, 2021). Thus, in current study we focused to evaluate MPHOSPH8 (MPP8) expression in Iraqi cancer patients, which is characterized as candidate gene to repress L1Hs transcription. MPP8 gene is a subset of HUSH complex (Liu *et al.*, 2018; Timms *et al*., 2016). That acts to repress LHs mobilization via heterochromatin formation. MPP8 has a critical role in several processes of cell life (Chang *et al*., 2011; Nishigaki *et al*., 2013) in mitosis stage of cell cycle, MPP8 gene regulates the transcription levels. However, in vitro studies , it has been identified that knockout MPP8 significantly exhibit colon tumor cell lines and reduced its growth (Liang *et al*., 2017). MPP8 was observed to be up regulate in lung

cancer cells and breast cancer (Matsumoto-Taniura *et al*., 1996). MPP8 inhibition significantly suppressed colony formation and proliferation, repressed invasion and promoted apoptosis of gastric cancer cells. In addition, MPP8 silencing increased Bax, p53 and PARP apoptosis proteins (Wang *et al*., 2020). Wang *et al*. (2020) out findings observed that MPP8 might be have as an oncogene via positive regulation function of gastric tumor cells through related cell death proteins and signaling pathways (Wang *et al.,* 2020). MPP8 repression in vivo and vitro studies inhibited metastasis and growth melanoma cancer (Yuan *et al*., 2017). According to implicate L1Hs and MPP8 gene in cancer progression current assay aimed to assess the correlation between L1Hs and MPP8 gene and recognize which cancer expressed these genes more than other types via evaluating expression levels of L1Hs and MPP8 genes in extracted RNA of blood samples of Iraqi patients of cancer comparing with healthy group.

MATERIALS AND METHODS

Samples collections Blood samples collection were taken from venous (5ml) for each sample from both cancer patients (60 individuals) and healthy individual (60) . The blood samples were collected in EDTA tube and transported in cool box to the laboratory for RNA extraction.

RNA extraction Whole RNA was extracted from collected blood samples of both patients and control groups via RNeasy kit from Qiagen and treated using DNase (Ambion AM 1907) following the instructions of manufacture. Then treated RNA were eliquaed and kept at -20ºc until use.

CDNA creation 1000ng of treated RNA samples were revers transcripted following manufactures instructions (superscript II breverse transcriptase) from thermo fisher scientific company and using random primers. Reactions free from reverse transcriptase were usually prepared and used in real time PCR to make double check there is no DNA was remaining . water free nuclease was used to dilute cDNA . cDNA eliquated and kept of -20ºc until used.

Gene expression quantification mRNA levels of L1Hs and MPP8 gene were quantified using qPCR using real time PCR system ABI 7500) from applied bio system company of master mix following the instruction SYBR green from life technology was used . GAPDH gene was severed as an interior control. Fold change was assessed using $-\Delta\Delta C_T$ method according to Livak and Schmittgen (2001) method.

Statistical analysis Graph pad prism software (version 8) was employed significant statistical was determined by utilizing student t test (unpaired). The data were showed with error bars to show the mean of standard error (SEM) or standard deviation (SD) . the significantly was determined if p value was less than 0.05 or equal.

RESULTS

Gene expression level of L1Hs and MPP8 genes was measured using real time PCR. L1Hs was significantly expressed in blood samples of patients compared with healthy group and cycle threshold (C_T) was 17.23±0.57 of cancer patients compared with C_T of healthy group which is 27.80±0.51 and less threshold cycle is more expression table 1 cycle threshold (C_T) of MPP8 gene of cancer patients was 27.65±0.61 at $p \leq 0.01$ while C_T value of healthy group was 30.79±1.08 (Table 1). L1Hs expression was up regulated 2.02 times in blood samples of patients compared with control group as represented in Table (1) and Figure (1). mRNA level of MPP8 gene was significantly increased approximately two times 1.98 in patients samples compared with control group as reviewed in Table (1) and Figurer (1).

Table (1) RT-qPCR assay analysis of studied gene L1Hs and MPP8. Ct refers to cycle threshold, ΔC_T mean represent C_T of control (GAPDH) – C_T of cancer samples of interested gene, $\Delta\Delta C_T$ results from ΔC_T of patients group subtraction from ΔC_T of interested gene from ΔC_T of healthy group of studied gene.

Genes		C_T target	ΔC_T Mean	$\Delta\Delta C_T$	Folding change
L1Hs	Healthy	27.80±0.51	-9.45±0.39	-1.021	2.029
	Patients	17.23±0.57	-10.48±0.28*		
MPP8	Healthy	30.79±1.08	2.43±0.21	-0.988	1.983
	Patients	27.65±0.61	1.44±0.13**		

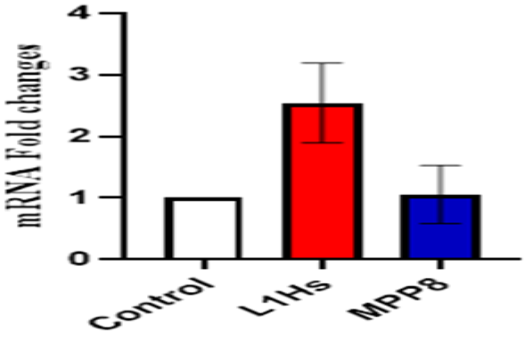

Figure (1) Fold change of mRNA level expression of inserted gene L1Hs and MPP8 of cancer samples compared with their levels in control group.

Table (2) showed folding change of L1Hs and MPP8 genes in several types of cancer. L1Hs expressed 5.68±1.12 in colon cancer samples compared with MPP8 level that is down regulate up to 1.75±0.05 at p≤0.01. Prostate cancer samples expressed L1Hs approximately five times 4.89±0.12 while expressed MPP8 at 1.64±0.30. There is no significant different in folding change between L1Hs and MPP8 genes in breast cancer 4.03±0.77 and 3.42±0.82 respectively. In cervical cancer, L1Hs up regulated three times 3.03±0.62 compared with MPP8 which is down regulated up to 1.04±0.80 at p≤0.05. There is no significant differ in folding change of L1Hs and MPP8 genes in bladder and ovary samples of cancer at p≤0.5913 and p≤0.245 respectively. However, the folding change of L1Hs was significantly increased up to 4.00±0.04 in tests cancer of blood samples compared with MPP8 which decreased up to 2.10±0.60 at p≤0.0001. Data in figure 3 showed a negative significant association between L1Hs transcript and MPP8 gene expression transcript (r = -0.4101).

Table (2) Fold change comparison between L1Hs and MPP8 genes for blood samples of cancer patients. P value (0.0001, 0.001) ns refers to no significant. Fold change transcript level of L1Hs compared with MPP8 transcript level change in several cancers including (colon, prostate, breast, cervical, bladder, ovarian and tests) cancers ns represents non-significant.

Type of cancer	Folding change L1Hs	Folding change MPP8	P value
Colon	5.68±1.12	1.75±0.05	0.0087
Prostate	4.81±0.12	1.64±0.30	0.0007
Breast	4.03±0.77	3.42±0.82	0.6209
Cervical	3.03±0.62	1.04±0.80	0.0216
Bladder	0.47±0.17	0.39±0.10	0.5913
Ovary	**1.35±0.32**	**0.99±0.10**	**0.2458**
Testis	**4.00±0.04**	**2.10±0.60**	**0.0001**

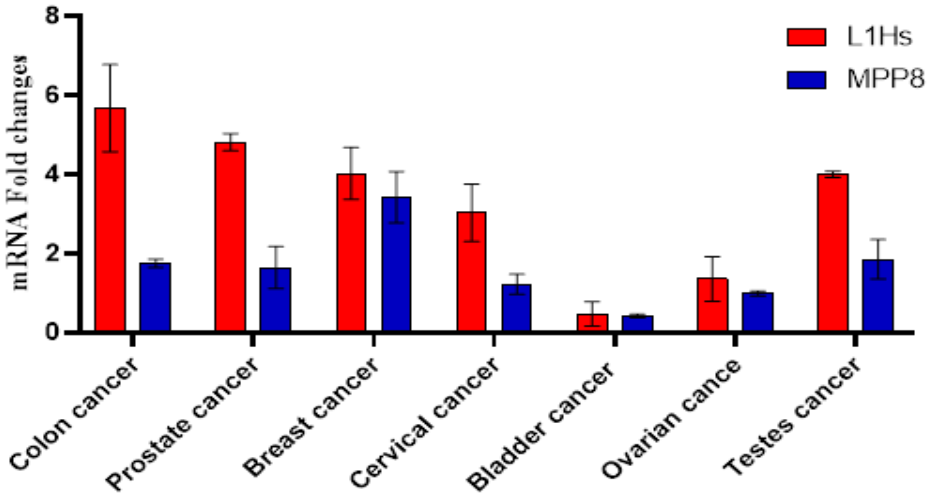

Figure (2) mRNA level in L1Hs gene compared with mRNA level of MPP8 gene in patients cancer cases.

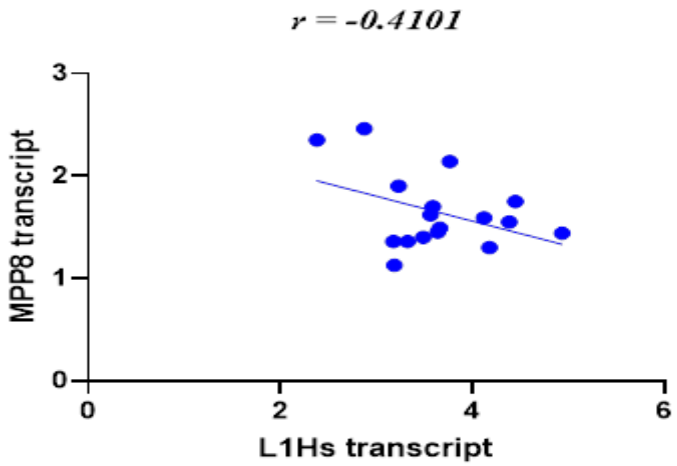

Figure 3 Correlation mRNA L1Hs expression and mRNA MPP8 expression in cancer patients.

DISCUSSION

L1Hs retrotransposone elements is mostly related to aging , genome development and diseases. L1Hs elements have a critical role in human genome instability (Pappalardo and Barra, 2021). Changing mRNA level in genomic material is often associated with several diseases including cancer (Ishak *et al*., 2016). Hundreds of novel insertions were observed among epithetical cancers including colorectal, gastric and pancreatic cancers and demonstrated these insertion in early phases of gastrointestinal cancer. L1Hs insertions enhance oncogene role in tumorgenesis pathway for example, L1Hs insertion can elevate mRNA level of ST18 gene and suggested to be an a critical contributor of hepatocellular cancer (Shukla *et al.,* 2013). Current study support the previous studies and find that line-1 expression level significantly raised in patients compared with its level in healthy group. L1Hs significantly expressed in patients of cancer compared with healthy. These results consistent with previous studies. Scott *et al.* (2016) characterized a novel insertion of a highly active L1 elements. This element disrupted one allele of Adenomatas polyposis coli (APC) gene and somatic allele mutation disrupt the other allele these two mutation caused losing function of APC gene and leading to cancer colon cancer (Scott *et al.*, 2016). L1Hs mobilization has been demonstrated in some tumors including lung, breast, colon, liver, ovarian, colorectal, prostate and gastrointestinal (Chen *et al*., 2006; Ewing *et al*., 2015; Solyom *et al.*, 2012). Thus, L1Hs element has different mechanisms to effect host gene expression. MPP8 significantly expressed in cancer patients samples at $p \leq 0.01$. these results corresponding with other studies that has been highlighted the role of MPP8 in metastasis and growth cancer cells (Yuan *et al*., 2017).MPP8 dawn regulation significantly increased the number of cells melanoma in S-phase without increasing number of cells in G0/G1 phase while the cell number was decreased in G2/M phase and causing reduced transcript level of some proteins that related cell cycle including CDK2 , cycling A and cycling B1. These findings refers to possible role of MPP8 knockdown to arrest cell cycle in S-phase of melanoma cancer (Yuan *et al*., 2017). A few studies explain a bout epigenetic regulation pathways to silence and control transcriptional level of DNA retrotransposon in cancer cell or in adults tissues of human (Cuellar *et al*., 2017; Kato *et al.*, 2018; Sheng *et al*., 2018; Tchasovnikarova *et al*., 2015; Tie *et al*., 2018). HUSH complex was characterized as a novel epigenetic regulate of L1Hs elements transcript and is responsible of integrity of human cells transgenes (Tunbak *et al*., 2020). Tissue culture studies suggested that L1Hs elements are silenced by two mechanisms including MPP8 and DNA methylation demonstrated that degradation MPP8 gene increased line-1 mRNA level (Müller *et al.*, 2021). The mechanism including that H3K9me3 recruited MPP8 by its chromdomain following by binding HUSH complex care and recruit SETDB1 causing additional prevalence of H3K9me3 and repress line-1 elements transcription (Müller *et al.*, 2021). There is no significant changes was identified in some cases of cancers including breast, bladder and ovary between L1Hs and MPP8 transcripts level genes as shown in table 2 and figure 2. The studies find down regulation in MPP8 expression level in some types of cancer and they demonstrated that could be related to DNA breaks occurs via L1Hs encoded endonuclease (Tunbak *et al*., 2020). Tunbak *et al.* (2020) showed that MPP8 was down regulated in several tumors and its depletion leads to damaging of DNA that mediated by L1Hs. We can conclude the L1Hs expressed significantly in cancer samples as well as there is a negative association between L1Hs and MPP8 and this study supported the study of (Liu *et al*., 2018).

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