Metabolic Brain Disease
https://doi.org/10.1007/s11011-019-00418-z

ORIGINAL ARTICLE

Hepatocellular carcinoma up-regulated long non-coding RNA: a putative marker in multiple sclerosis

Arezou Sayad[1] · Mohammad Taheri[2] · Shahram Arsang-Jang[3] · Mark C. Glassy[4] · Soudeh Ghafouri-Fard[1]

Received: 7 November 2018 / Accepted: 16 April 2019

Abstract

Highly up-regulated in liver cancer (*HULC*) is a cancer-associated long non-coding RNA (lncRNA) which may regulate expression of other genes by working as a competing RNA for microRNAs. In the current study, we assessed transcript levels of this lncRNA in peripheral blood of multiple sclerosis (MS) patients and healthy persons to evaluate its possible role in the pathogenesis of this inflammatory disease and its diagnostic power. The results of Multilevel Bayesian showed no significant difference between cases and controls ($P = 0.002$, 95% confidence interval (CI) = [3.08, 13.3]). However, based on the results of Quantile regression, there was a significant difference in *HULC* expression between cases and controls after controlling the effects of sex and age ($P = 0.002$, 95% CI = [3.08, 13.3]) which shows different trends in males and females. *HULC* expression was inversely correlated with age of male subjects but not female subjects. *HULC* transcript levels had 91.1% accuracy in diagnosis of MS disease (Specificity: 80%, Sensitivity: 86.6%). The diagnostic power of *HULC* was higher in male subjects aged less than 50 years (AUC = 0.923, Specificity: 80%, Sensitivity: 100%). The present study shows the possibility of application of transcript levels of *HULC* as diagnostic marker in MS disease. However, future studies with larger sample sizes are necessary to validate our results.

Keywords *HULC* · Multiple sclerosis · lncRNA

Introduction

The *highly up-regulated in liver cancer* (*HULC*) is a long non-coding RNA (lncRNA) that has been firstly identified as the most up-regulated gene in hepatocellular carcinoma (HCC) through screening and sequencing of a HCC-specific gene library. The role of this lncRNA in post-transcriptional regulation of gene expression has been verified via gene knockdown experiments (Panzitt et al. 2007). Afterwards, RNA affinity purification method has shown IGF2 mRNA-binding proteins (IGF2BPs) as specific binding partners of this lncRNA. Further studies have verified the role of IGF2BP1 in induction of *HULC* degradation (Hammerle et al. 2013). More recently, participation of *HULC* has been demonstrated in osteosarcoma. Notably, in vitro experiments have shown that *HULC* acts as an endogenous sponge for miR-122 (Kong and Wang 2018). *HULC* has also been shown to bind with miR-200a-3p (Jiang and Liu 2018). miR-200a might have a putative role in the regulation of immune response since a number of signaling proteins in the Toll like receptor 4 (TLR4) pathway has been recognized as probable targets for miR-200 family members (Wendlandt et al. 2012). In addition, miR-122, another partner of *HULC* has been identified among miRNAs with differential expression between RRMS patients and healthy subjects (Selmaj et al. 2017). Recent studies have reported down-regulation of *HULC* in peripheral blood mononuclear cells of relapsing-remitting (RR) and primary progressive (PP) multiple sclerosis (MS) patients compared with healthy subjects (Oldoni et al. 2017).

✉ Mohammad Taheri
mohammad_823@yahoo.com

✉ Soudeh Ghafouri-Fard
s.ghafourifard@sbmu.ac.ir

[1] Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[2] Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[3] Clinical Research Development Center (CRDU), Qom University of Medical Sciences, Qom, Iran

[4] Translational Neuro-Oncology Laboratory, UCSD Moores Cancer Center, La Jolla, CA, USA

Published online: 02 May 2019

Table 1 Nucleotide sequences of primers and probes used for the expression analysis

Gene name	Primer and probe sequence	Primer and probe length	Product length
HPRT1	F: AGCCTAAGATGAGAGTTC	18	88
	R: CACAGAACTAGAACATTGATA	21	
	FAM -CATCTGGAGTCCTATTGACATCGC- TAMRA	24	
HULC	F: ACGTGAGGATACAGCAAGGC	20	75
	R: AGAGTTCCTGCATGGTCTGG	20	
	FAM-CGTGACGACTCTTCCTGGCTTGCA-TAMRA	24	

Taken together, we hypothesized that transcript levels of *HULC* can be applied as diagnostic markers in MS patients. Consequently, we conducted the current study to compare expression of this lncRNAs between RRMS patients and age −/sex-matched healthy subjects and assess diagnostic power of *HULC* expression levels in this disorder.

Material and methods

Study participants

A total of 50 RRMS patients (Female/ Male: 35 (70%) / 15 (30%), Age (mean ± SD): 36.2 ± 2.7) and 50 healthy subjects with the same sex ratio (Age (mean ± SD): 35.3 ± 2.4) participated in the current study. Age at disease onset and disease duration (mean ± SD) in case group was 31.41 ± 2.8 and 4.58 ± 3.2 respectively. Expanded Disability Status Scale (EDSS) score of patients (mean ± SD) was 3.07 ± 2.5. All patients were responsive to IFN-β (CinnoVex, Cinagene Company, Iran) and were in remission in the last 3 months before sampling. People registered in the control group were healthy volunteers without any neurological or inflammatory disorders. The study protocol was approved by the Ethical Committee of Shahid Beheshti University of Medical Sciences. Written informed consents were obtained from all participants.

Expression study

Five milliliters of venous blood samples were collected from study participants. RNA was isolated from these specimens using Hybrid-RTM blood RNA extraction Kit (GeneAll, Seoul, Korea). After verification of RNA quantity and quality with NanoDrop 2000 (Thermo Fisher Scientific), cDNA was synthesized from all samples using High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Gent, Belgium). TaqMan® Universal PCR Master Mix (ThermoFisher Scientific, Gent, Belgium) was used for quantification of *HULC* levels. *HPRT1* gene was used as internal control. The primers and probes sequences and PCR product length are shown in Table 1.

Statistical analyses

The differences in mean values of *HULC* expression between two groups were assessed using Multilevel Bayesian model. The observation effects were considered as random in the analysis model. The t student/Gaussian prior distribution was assumed for parameters with 8000 iteration and 1000 warm-up. The effects of potential confounding variables were measured through application of Quantile regression. The Box-Cox transformation was used for normalization of the data. The model was established using Stan packages in R 3.5.1 environment. For all statistical analyses, $P < 0.05$ was considered as significant. We also schemed the sensitivity

Table 2 Multilevel Bayesian results of association between *HULC* expression and disease (RE: relative expression, *P* values were estimated from Frequentist method)

HULC expression		Controls number	Patients number	Posterior RE difference	SE	*P* value	95% credible intervals for RE difference
Total		50	50	1.63	1.09	0.002	[−0.52, 3.74]
Male		15	15	−8.862	2.44	0.034	[−13.71, −3.99]
Female		35	35	1.634	1.08	0.161	[−0.48, 3.74]
≤50	Male	5	13	−9.781	3.03	0.005	[−15.75, −3.18]
	Female	27	30	0.914	1.23	0.776	[−1.45, 3.38]
>50	Male	10	2	−7.623	3.94	0.821	[−15.38, 0.52]
	Female	8	5	5.09	2.56	0.05	[0.1, 10.47]

Table 3 The results of Quantile regression for controlling the effects of age and sex (Control group was regarded as reference)

Variable	Beta	SE	t	*P* value	95% CI
Group (Case/Control)	8.19	2.57	3.18	0.002	[3.08, 13.3]
Sex	4.12	2.30	1.79	0.076	[−0.44, 8.68]
Age	0.06	0.06	1.07	0.286	[−0.05, 0.18]
Group*Sex	−10.07	2.98	−3.39	0.001	[−15.98, −4.17]

versus 1-specifity using MedCalc Statistical Software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2016) and calculated the area under the curve (AUC) to assess diagnostic power of *HULC* in MS.

Results

Based on the results of Multilevel Bayesian analysis, *HULC* expression was not different between cases and controls (95% Credible Intervals (CrI): −0.52, 3.74)) (Table 2). Based on the results of Quantile regression (Table 3), a sex-based difference has been detected in gene expression between cases and controls. Subgroup analysis showed significant difference in male subjects (95% CrI: −13.7, −3.9) but not female subjects (95% CrI: −0.48, 3.74). In addition, Table 2 shows that difference in *HULC* gene expression is significant between patients and healthy controls not only for men under 50 but also for women over 50 years old (although in general for women without age distribution these differences they are not significant). Figure 1 depicts the results of expression analysis.

Based on the results of Quantile regression and after controlling the effects of sex and age, there was a significant difference in *HULC* expression between cases and controls ($P = 0.002$, 95% CI = [3.08, 13.3]) (Table 3).

HULC expression was inversely correlated with age of male subjects but not female subjects (Table 4).

Based on the AUC values, *HULC* transcript levels had 91.1% accuracy in diagnosis of MS disease (Specificity: 80%, Sensitivity: 86.6%) (Fig. 2). The diagnostic power of *HULC* was higher in male subjects aged under 50 (AUC = 0.923, Specificity: 80%, Sensitivity: 100%) (Fig. 3).

Discussion

In the present study, we demonstrated different expression levels of *HULC* in peripheral blood of MS patients compared with healthy subjects. This lncRNA has been firstly detected in blood of HCC patients and has been suggested as a potential biomarker in these patients (Panzitt et al. 2007). More recently, *HULC* dys-regulation in MS patients has been reported through lncRNA profiling in 5 patients with RRMS, 5 with PPMS and 5 age matched controls and further validation of the preliminary results in a cohort of Italian MS patients (Oldoni et al. 2017). Consistent with the latter study, we demonstrated significant down-regulation of *HULC* in male MS patients compared with healthy subjects. Although the exact function of *HULC* in the process of inflammation has not been recognized yet, this lncRNA has an established role in sponging some miRNAs such as miR-122 (Kong and Wang 2018). miR-122 has been shown to be significantly lower in serum of MS patients in both relapse and remission phases compared with healthy subjects (Selmaj et al. 2017). This miRNA has also an anti-inflammatory effect in liver tissue (Hsu et al. 2012). Consequently, other mechanisms rather than miR-122 sponging must be involved in the pathogenic role of *HULC* down-regulation in MS patients. *HULC* also activates the miR-200a-3p/ZEB1 signaling pathway (Li et al. 2016). While miR-200 family members are involved in the

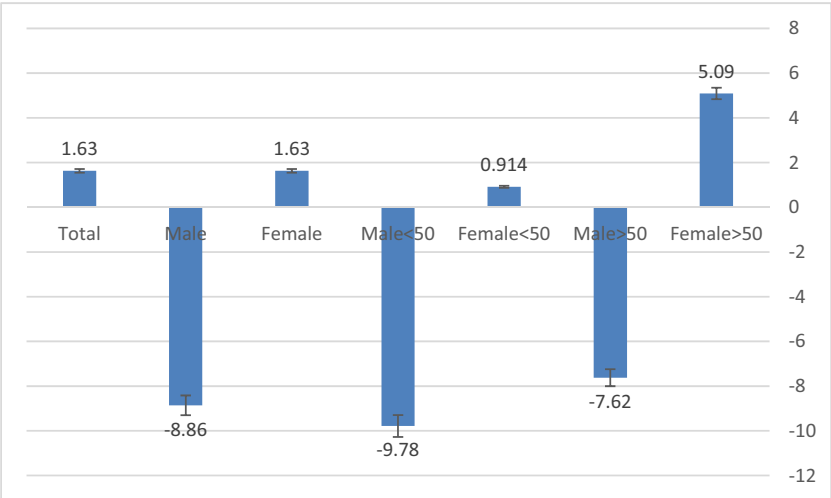

Fig. 1 The results of expression of *HULC* in different subgroups of patients compared with corresponding control subgroups

Table 4 Spearman correlation coefficients between *HULC* expression and other variables (**Correlation is significant at the 0.01 level)

	Group		Gender	
	Case	Control	Male	Female
Age	−0.054	−.114	−.488**	−.011

regulation of TLR4 pathway (Wendlandt et al. 2012), ZEB1 has been recognized as a neuroprotective protein (Bui et al. 2009). So this cascade can be involved in *HULC* role in MS. Consistent with this hypothesis, acute exposure to CSF sample of MS patients has decreased expression of *Zeb1* in cultured rat neurons (Vidaurre et al. 2015). It is worth mentioning that based on the small numbers of individuals in each sex- and age-based subgroup; it is difficult to draw specific conclusions. So these results should be verified in larger samples of male subjects.

The observed sex-determined down-regulation of *HULC* in MS patients is in line with the previous reports regarding the role of sex hormones in both pathogenesis of MS and pateints' response to treatments (Nicot 2009). We also detected inverse correlation between *HULC* expression and age only in male subjects which might imply the existence of sex-determined regulatory mechanism for *HULC* expression. However, due to small number of patients in this group, such observation should be interpreted with caution.

Finally, we assessed diagnostic power of *HULC* in total subjects and in subgroups of study participants. *HULC* transcript levels had 91.1% accuracy in diagnosis of MS disease in total subject. Notably, the diagnostic power of *HULC* was 92.3% in male subjects aged less than 50 which is consistent with the observed high level of down-regulation of *HULC* in this patients' subgroup. Consequently, *HULC* expression could be used as MS marker especially in male subjects aged less than 50.

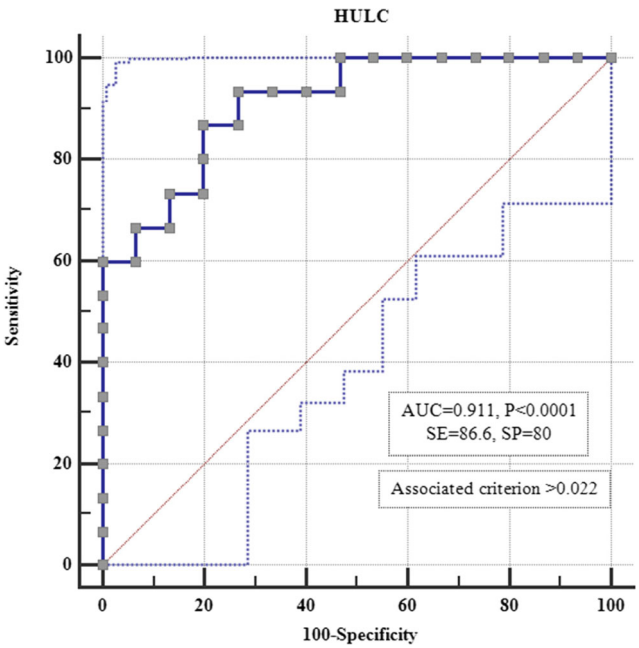

Fig. 2 ROC curve analysis for assessment of *HULC* diagnostic power in total subjects

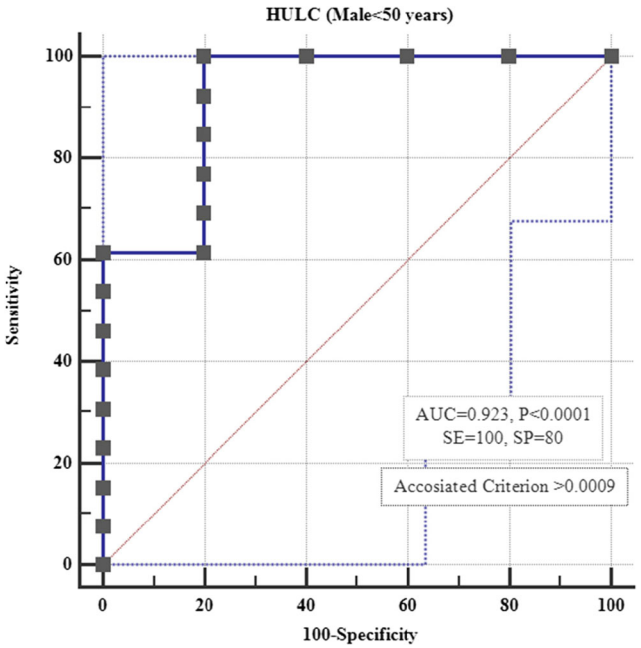

Fig. 3 ROC curve analysis for assessment of *HULC* diagnostic power in male subjects aged less than 50

In brief, we demonstrated down-regulation of *HULC* in male MS patients compared with healthy subjects and reported suitability of its transcript levels as diagnostic markers for MS disease. Future studies with larger sample sizes are needed to confirm our results.

Acknowledgements The current study was supported by a grant from Shahid Beheshti University of Medical Sciences.

Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

References

Bui T, Sequeira J, Wen TC, Sola A, Higashi Y, Kondoh H, Genetta T (2009) ZEB1 links p63 and p73 in a novel neuronal survival pathway rapidly induced in response to cortical ischemia. PLoS One 4: e4373. https://doi.org/10.1371/journal.pone.0004373

Hammerle M et al (2013) Posttranscriptional destabilization of the liver-specific long noncoding RNA HULC by the IGF2 mRNA-binding protein 1 (IGF2BP1). Hepatology (Baltimore, Md) 58:1703–1712. https://doi.org/10.1002/hep.26537

Hsu SH, Wang B, Kota J, Yu J, Costinean S, Kutay H, Yu L, Bai S, la Perle K, Chivukula RR, Mao H, Wei M, Clark KR, Mendell JR,

Caligiuri MA, Jacob ST, Mendell JT, Ghoshal K (2012) Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. J Clin Invest 122:2871–2883. https://doi.org/10.1172/jci63539

Jiang Z, Liu H (2018) Metformin inhibits tumorigenesis in HBV-induced hepatocellular carcinoma by suppressing HULC overexpression caused by HBX. J Cell Biochem 119:4482–4495. https://doi.org/10.1002/jcb.26555

Kong D, Wang Y (2018) Knockdown of lncRNA HULC inhibits proliferation, migration, invasion, and promotes apoptosis by sponging miR-122 in osteosarcoma. J Cell Biochem 119:1050–1061. https://doi.org/10.1002/jcb.26273

Li SP, Xu HX, Yu Y, He JD, Wang Z, Xu YJ, Wang CY, Zhang HM, Zhang RX, Zhang JJ, Yao Z, Shen ZY (2016) LncRNA HULC enhances epithelial-mesenchymal transition to promote tumorigenesis and metastasis of hepatocellular carcinoma via the miR-200a-3p/ZEB1 signaling pathway. Oncotarget 7:42431–42446. https://doi.org/10.18632/oncotarget.9883

Nicot A (2009) Gender and sex hormones in multiple sclerosis pathology and therapy. Front Biosci-Landmrk 14:4477–4515. https://doi.org/10.2741/3543

Oldoni E et al (2017) Long non coding RNA expression profile in Peripheral Blood Mononuclear Cells from multiple sclerosis patients: potential biomarkers of disease susceptibility and progression. In: Multiple Sclerosis Journal, 2017. Sage Publications LTD 1 Olivers Yard, 55 City Road, London EC1Y 1SP, England, pp 580–581

Panzitt K, Tschernatsch MMO, Guelly C, Moustafa T, Stradner M, Strohmaier HM, Buck CR, Denk H, Schroeder R, Trauner M, Zatloukal K (2007) Characterization of HULC, a novel gene with striking up-regulation in hepatocellular carcinoma, as noncoding RNA. Gastroenterology 132:330–342. https://doi.org/10.1053/j.gastro.2006.08.026

Selmaj I, Cichalewska M, Namiecinska M, Galazka G, Horzelski W, Selmaj KW, Mycko MP (2017) Global exosome transcriptome profiling reveals biomarkers for multiple sclerosis. Ann Neurol 81:703–717. https://doi.org/10.1002/ana.24931

Vidaurre OG et al (2015) Cerebrospinal fluid ceramides from patients with multiple sclerosis impair neuronal bioenergetics (vol 137, pg 2271, 2014). Brain 138:e367. https://doi.org/10.1093/brain/awv090

Wendlandt EB, Graff JW, Gioannini TL, McCaffrey AP, Wilson ME (2012) The role of microRNAs miR-200b and miR-200c in TLR4 signaling and NF-kappaB activation. Innate Immunity 18:846–855. https://doi.org/10.1177/1753425912443903