Novel Role of GDF15 in non-alcoholic steatohepatitis

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Abstract

Growth differentiation factor (GDF) 15) is known as a stress-induced circulating cytokine and is correlated with cancer of many types, cardiovascular and kidney diseases. We enrolled a cross- sectional obese cohort of 152 patients who underwent bariatric surgery in the First Affiliated Hospital of Jinan University from 2017 to 2019. Clinical parameters and biochemical markers for liver injury were measured. Stage of NAFLD is accessed by liver histology using NAFLD Activity Score (NAS). We also established a diet-induced animal model for NASH with the use of choline deficient and methionine restricted L-amino acid diet with 60 kcal% fat (CDAHF60). In both human and mouse study, there is a stepwise increase in the serum level of GDF15 during the progression of NASH and is the most significantly associated with steatosis when compared to inflammation, ballooning and fibrosis. Tissue distribution shows that liver is the major tissue for increased GDF15 expression while liver fractionation reveals that hepatocytes are the dominant source of induced GDF 15 expression in liver. Flow cytometry analysis identifies Kupffer cell which expresses the receptor and co-receptor of GDF15 as the potential target cell of GDF15 actions under NASH.

Introduction

Growth differentiation factor (GDF) 15, a divergent member of the transforming growth factor-β superfamily, is synthesized as an inactive precursor, which is subsequently cleaved and secreted as a disulfide-linked mature protein with a molecular weight of 24.5 kDa. GDF15 is known as a stress-induced cytokine and is correlated with cancer, cardiovascular and kidney diseases. However, the role of GDF15 in non-alcoholic steatohepatitis (NASH) remains unclear.

Methodology

1. Human study (HS): We enrolled a cross- sectional obese cohort who underwent bariatric surgery in the First Affiliated Hospital of Jinan University from 2017 to 2019. The following exclusion criteria were applied: (i) With hepatitis B virus infection; (ii) Age < 18 years old; (iii) non-NAFLD. A total of 152 patients were included in our study. Clinical parameters and biochemical markers for glucose and lipid metabolism and liver injury were measured. Hepatic fat content was determined by Magnetic Resonance Imaging (MRI). Stage of NAFLD is accessed by liver histology using NAFLD Activity Score (NAS).
2. Mouse Study (MS): We established a diet-induced animal model for NAFLD with the use of choline deficient and methionine restricted L-amino acid diet with 60 kcal% fat (CDAHF60). Dynamic changes of serum GDF15 levels during the development of NAFLD were measured and tissue source as well as cell source of GDF15 is explored by real-time PCR, western blotting and immuno-histological staining and explant culture. The potential target cell in liver of GDF15 actions is analyzed by flow cytometry.

Result

1. Serum GDF15 level was positively correlated with liver injury markers

Table1.Clinical and metabolic characteristics of human subjects

Parameters	Normal (n=16)	Steatosis (n=31)	B. NASH (n=62)	NASH (n=43)	p-value
Age	27.75 ± 1.78	31.48 ± 1.60	28.49 ± 0.85	27.31 ± 1.00	0.092
Sex(M:F)	2(14)	13(18)	29(33)	21(22)	0.070
BMI	33.78 ± 0.99	40.50 ±1. 68	40.88 ± 1.01	42.98 ± 1.35	0.003
Neck Cir	37.43 ± 0.77	42.84 ± 0.80	43.42 ± 0.64	44.52 ± 0.81	0.000
Waist_Cir	106.88 ± 2.71	125.53 ± 3.31	124.69 ± 1.91	127.73 ± 2.87	0.000
Hip_Cir	117.28 ± 1.96	126.54 ± 2.97	126.12 ± 1.67	131.92 ± 2.26	0.005
SBP	118.81 ± 2.73	130.55 ± 2.55	129.27 ± 1.70	130.60 ± 2.51	0.032
DBP	77.81 ± 2.74	81.84 ± 2.11	81.53 ± 1.51	86.21 ± 2.46	0.122
TCHOL	5.01 ± 0.17	4.98 ± 0.20	5.06 ± 0.13	5.23 ± 0.16	0.739
TG	1.28 ± 0.11	2.93 ± 1.27	2.46 ± 0.25	2.84 ± 0.52	0.521
HDL-C	1.22 ± 0.06	1.08 ± 0.05	1.00 ± 0.03	0.96 ± 0.03	0.001
LDL-C	3.06 ± 0.13	2.90 ± 0.14	3.09 ± 0.09	3.16 ± 0.12	0.498
Glucose	5.09 ± 0.12	6.40 ± 0.57	6.84 ± 0.41	6.72 ± 0.42	0.195
HbA1c	5.35 ± 0.09	6.06 ± 0.19	6.54 ± 0.23	6.53 ± 0.24	0.027
Insulin	14.28 ± 1.96	21.38 ± 1.87	23.42 ± 1.70	31.74 ± 3.05	0.000
C-Peptide	2.36 ± 0.19	3.75 ± 0.25	3.78 ± 0.14	4.76 ± 0.31	0.000
HOMA-IR	3.29 ± 0.48	5.88 ± 0.64	7.13 ± 0.68	9.77 ± 1.09	0.000
ALT	18.71 ± 1.99	50.63 ± 8.68	72.95 ± 7.27	73.86 ± 8.10	0.001
AST	17.88 ± 0.79	30.93 ± 3.78	41.52 ± 3.53	48.70 ± 5.40	0.001
APOB	0.96 ± 0.04	0.98 ± 0.04	1.08 ± 0.03	1.09 ± 0.04	0.083
GDF15	134.97 ± 9.99	188.68 ± 20.14	197.43 ± 13.46	236.35 ± 22.08	0.024

Table 2. Correlation of serum GDF15 levels with liver injury markers

Parameters	Pearson's Coefficient®	P value
Age	0.106	0.199
BMI	0.221	0.006 **
TG	0.152	0.061
TCHOL	0.027	0.745
HDL-C	-0.203	0.012 *
LDL-C	-0.052	0.522
Glucose	0.164	0.044 *
HbA1c	0.178	0.029 *
Insulin	0.226	0.005 **
C-Peptide	0.310	0.000 ***
HOMA-IR	0.297	0.000 ***
ALT	0.195	0.016 *
AST	0.327	0.000 ***
ApoB	0.078	0.341
Ferritin	0.277	0.001 **

2. There was a stepwise increase in the serum GDF15 level during the progression of NAFLD in both human and mouse study.

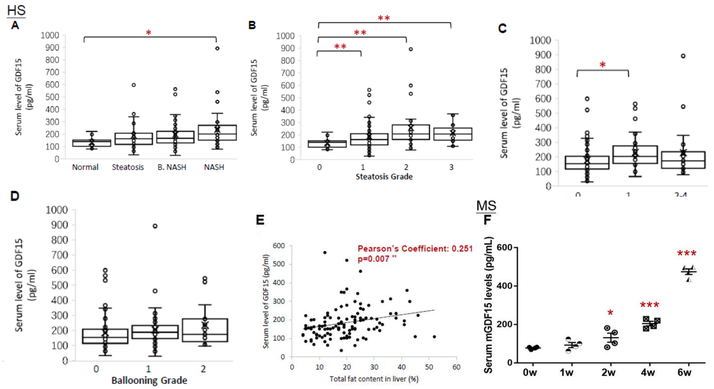

Figure1. Correlation of serum GDF15 levels with NAFLD in human and mice. (A-E)Human Study (HS): (A) Serum GDF15 levels in patients with NAFLD in which the liver biopsy was evaluated and scored as simple steatosis versus borderline NASH versus NASH.(B-D)Serum GDF15 levels after scoring liver biopsies for (B)steatosis, (C)inflammation, (D)hepatocyte ballooning. (E) Correlation of serum GDF15 levels with fat content in liver as measured by non-invasive quantitative method susing MRI in NAFLD patients. (F) Mouse Study (MS): Serum GDF15 levels in wild-type mice fed with CDAHF60 for various time periods.

3. Liver contributes to the induced GDF 15 expression and secretion under NASH.

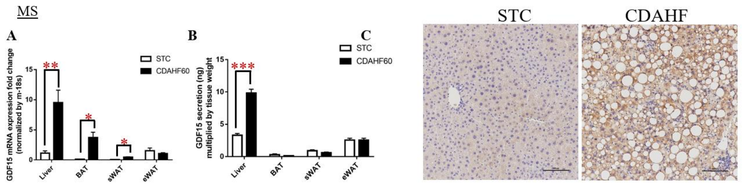

Figure2. (A-C) MS: (A) GDF15 mRNA expression in liver, BAT, sWAT and eWAT isolated from healthy or NASH mice. mRNA is presented as fold expression (mean ± SEM) relative to the STC fed mice from liver (set at 1) and normalized to the geometric mean of 18s gene expression. (B) GDF15 secretion from in liver, BAT, sWAT and eWAT isolated from mice fed with STC or CDAHF60 in explant culture. GDF15 levels are presented as multiplication of total tissue weight. (C) Hepatic GDF15 expression was detected by IHC staining of liver sections from healthy and NASH mice.

4. Macrophage is the potential target cell of GDF15 in NASH

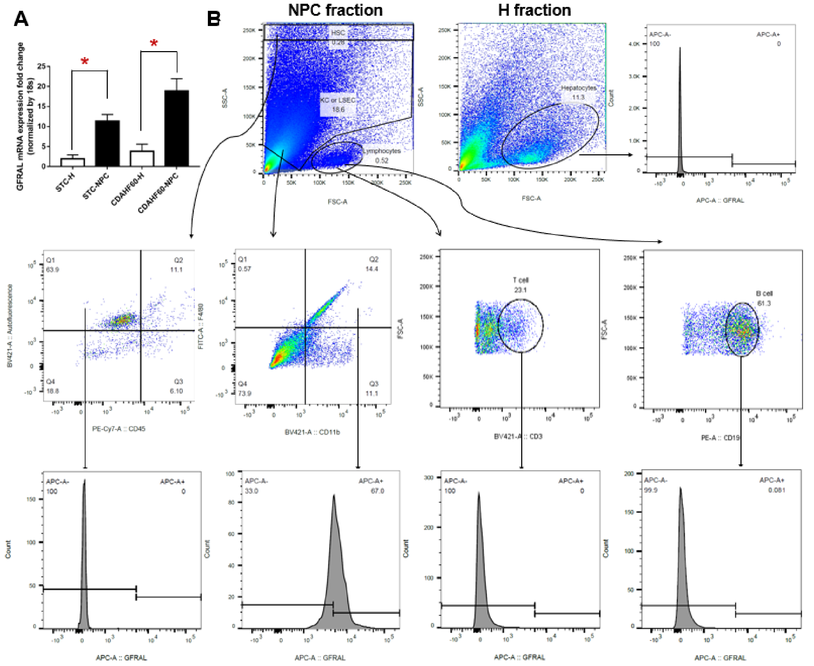

Figure 3. Macrophages are the potential target for GDF15 actions in NASH. (A) Liver from mice fed with STC or CDAHF60 were fractionated into hepatocyte enriched fraction and NPC enriched fraction using collagenase digestion and differential centrifugation. GFRAL mRNA expression is presented as fold expression (mean ± SEM) relative to the hepatocytes of STC fed mice (set at 1) and normalized to the geometric mean of 18s gene expression. *p < 0.05. (B) NPC and hepatocytes enriched fraction are subjected to flow cytometry analysis, respectively. In NPC fractions, HSCs are gated as CD45 negative, autofluorescence positive cells. Macrophages are gated as CD45 positive, CD11b positive, F4/80 positive cells. T cells are gated as CD45 positive, CD3 positive cells. B cell are gated as CD45 positive, CD19 positive cells. In hepatocyte-enriched fraction, hepatocytes are further gated according to FSC and SSC. GFRAL expression of HSCs, Macrophages, T cells, B cells and hepatocytes are presented. All figures are representatives of 2-3 experiments. HSCs, hepatic stellate cells. KCs, Kupffer cells. FSC, forward scatter. SSC, side scatter.

Conclusion

Serum GDF15 levels are closely associated with progression of NAFLD in both human and mice. Macrophage is the potential target cell of GDF15 fuction in NASH.