GC-MS-based metabolomics analysis of 3,2-trans-enoyl-CoA isomerase knockdown mutants in human hepatoma cells

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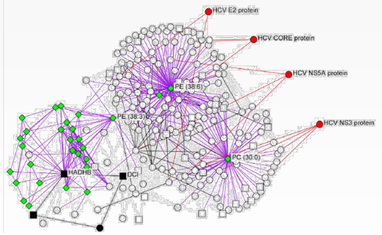
Overview

- A previous computational model predicted that 3,2-trans-enoyl-CoA isomerase (DCI) is an essential bottleneck protein regulating overall reprogramming of cellular metabolism during hepatitis C virus (HCV) infection.
- To gain a preliminary understanding of the effect of DCI on global cellular metabolism, we performed GC-MS analyses of wild type and DCI knockout cells.

Introduction

- HCV infection remains the leading cause of chronic hepatitis in the United States.
- A recent proteomics and lipidomics study of HCV-infected human hepatoma (Huh-7.5) cells identified DCI as a bottleneck node in the host response to HCV infection, based on a protein-lipid association network analysis[1].
- DCI is a key enzyme involved in fatty acid oxidation.
- To investigate the role of DCI in the HCV life cycle, we analyzed DCI knockdown mutants in Huh-7 cells using GC-MS-based metabolomics.

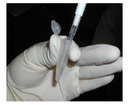


Several key bottlenecks identified by computational modeling efforts[1].

- lipidomics-proteomics correlation
- protein-protein interactions
- ♦ lipid species
- ● HCV proteins
- ■ proteins involved in fatty acid oxidation

Methods

Sample preparation



Wild type Huh-7 cells, a shRNA non-targeting control, and complete and incomplete DCI knockdown mutants (DCI-1 and DCI-3, respectively), 6 biological replicates each

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Cells washed, pelleted and snap-frozen.

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Extraction with chloroform/methanol (2:1, v/v)

↓

Derivatization using methoxyamine and N-methyl-N-(trimethylsilyl) trifluoroacetamide

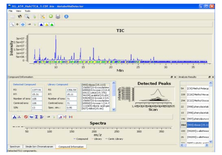
Data acquisition

Column: HP-5MS (30 m ×0.25 mm ×0.25 µm, Agilent)

Program: 60°C for 1 min, ramp to 325°C by10°C/min, hold at 325°C for 5 min

Detection: Agilent GC 7890A with MSD 5975C

Data analysis



Data-Preprocessing: Metabolite Detector for peak alignment, deconvolution, and identification using the Fiehn Metabolomics RTL Library

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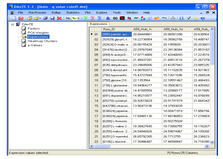
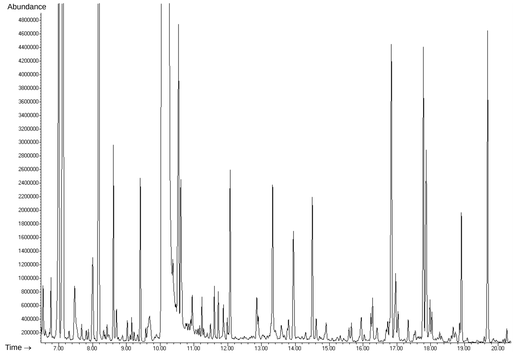
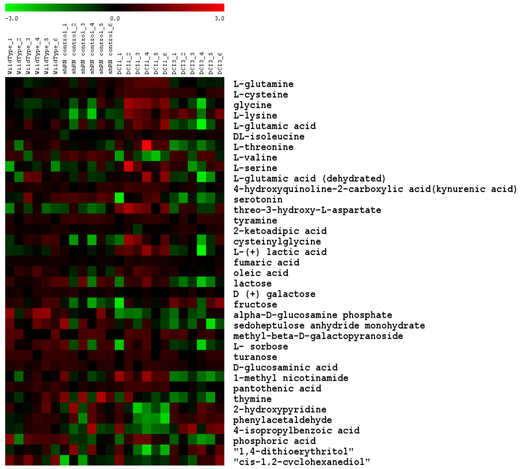


Statistical analysis: DAnTE for ANOVA, PCA and heatmap

Results[2]



Representative GC-MS chromatogram of Huh-7 cell extracts.



DCI deficiency causes pronounced alterations in the cellular metabolome. 37 metabolites demonstrated significant differences in abundance by ANOVA (q<0.05). Shown are z-score transformed metabolite abundances.

64 metabolites displaying qualitative differences were identified in the DCI-1 knockdown cells compared to the controls based on a G-test (p<0.05).

Metabolite	PubChem ID	G test P-Values	Wild Type	shRNA control	DCI 1	DCI 3
L-methionine	6137	0.0000	0	0	6	0
arabitol	94154	0.0000	0	0	6	1
dihydroxyacetone phosphate	668	0.0003	0	1	6	2
1-hexadecanol	2682	0.0007	4	3	6	0
alpha-glucosamine 1-phosphate	740	0.0010	6	6	1	5
L-serine	5951	0.0010	0	1	5	0
3-hydroxypropanoic acid	68152	0.0020	5	5	0	2
5-aminoimidazole-4-carboxamide 1	9679	0.0046	3	4	0	0
aspartic acid 1	5960	0.0055	5	3	6	1
(+)-6-aminopenicillanic acid	11082	0.0061	4	5	0	4
adipic acid	196	0.0061	2	2	1	6
D-ribose-5-phosphate	439167	0.0061	4	2	5	0
glycyl-L-tyrosine	92829	0.0061	5	4	4	0
2-ketoadipic acid	71	0.0070	2	0	5	1
L-glutamine	738	0.0077	4	0	3	5
N-methylalanine	5288725	0.0077	4	3	0	5
D-threitol	169019	0.0084	4	6	6	2
N-carbamyl-L-glutamic acid	3679006	0.0084	0	2	4	0
acetol	8299	0.0092	6	5	2	6
aspartic acid 2	5960	0.0092	4	0	0	1
hypoxanthine	790	0.0092	1	0	4	0
N-acetyl-L-aspartic acid	65065	0.0092	0	1	4	0
allo-inositol	892	0.0105	3	5	0	2
glycerol 1-phosphate	754	0.0105	2	3	5	0
lactulose	11333	0.0105	0	5	2	3
caprylic acid	379	0.0113	4	4	1	0
galacturonic acid	439215	0.0113	4	1	0	4
alpha-D-glucosamine 1-phosphate	740	0.0114	3	5	0	3
guanosine-5'-monophosphate	6804	0.0207	0	0	3	0
L-asparagine	236	0.0207	0	0	3	0
biuret 1	7913	0.0214	4	2	0	4
2-hydroxyquinoline	6038	0.0262	1	5	5	2
4-hydroxyphenylacetic acid	127	0.0262	2	5	1	5
cytosine	597	0.0269	3	5	2	6
dehydroascorbic acid	835	0.0269	6	5	2	3
D-glucosaminic acid	73563	0.0269	6	3	5	2
nicotinoylglycine	68499	0.0269	4	3	0	1
1-methyl-L-tryptophan 1	676159	0.0350	2	0	0	3
4-vinylphenol	62453	0.0350	2	0	0	3
biuret 2	7913	0.0350	3	2	0	0
2,3-dimethylsuccinic acid	11848	0.0361	5	6	2	5
3-(2-hydroxyphenyl)propanoic acid	873	0.0361	0	1	4	1
4-nitrocatechol	3505109	0.0361	0	1	4	1
O-phosphocolamine	1015	0.0361	1	1	4	0
benzene-1,2,4-triol	10787	0.0404	5	4	6	2
gluconic acid	71	0.0404	2	5	6	4
1-hydroxy-2-naphthoic acid	6844	0.0428	3	4	2	0
3-hydroxyphenylacetic acid	12122	0.0428	3	4	0	2
gluconic acid lactone	736	0.0428	2	0	4	3
N-acetyl-L-glutamic acid	70914	0.0428	2	4	3	0
phenylethylamine	1001	0.0428	2	4	3	6
15-keto-prostaglandin F-2-alpha	5280887	0.0481	1	0	0	3
2-furoic acid	6919	0.0481	0	3	0	1
4-hydroxybenzoic acid	135	0.0481	1	0	0	3
8-aminocaprylic acid	66085	0.0481	1	3	0	0
glycerol	753	0.0481	6	5	6	3
L-threonine	6288	0.0481	5	3	6	6
mandelic acid	439616	0.0481	1	0	3	0
N- carbobenzoxy-L-leucine	74840	0.0481	0	3	1	0
pantothenic acid	6613	0.0481	6	5	6	3
ribose	993	0.0481	0	1	3	0
D-glucose-6-phosphate	439958	0.0482	1	2	5	1
maltitol	16217663	0.0482	5	4	5	1
N-acetyl-ornithine	439232	0.0482	2	1	5	1

Conclusions

- 732 features were identified by matching the GC-MS data to entries in the Fiehn Metabolomics RTL Library.
- 37 metabolites were identified as having significant differences (q < 0.05) in abundance among the four cell types based on ANOVA, while a G-test analysis identified an additional 64 metabolites as significantly different (p < 0.05) based on occurrence.
- Most amino acids and their derivatives, fatty acids, sugars, and vitamins were increased in DCI-1 knockdown cells relative to the other three cell types, while nucleobases and aromatic metabolites were decreased.
- Increases in amino acids and intermediates of urea synthesis are consistent with multiple fatty acid oxidation deficient mouse models and suggest an increased dependence on amino acids for energy production in the absence of mitochondrial β-oxidation of fatty acids.

Acknowledgements

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