“Sterol Regulatory Element Binding Proteins: Master Regulators of Lipid and Lipoprotein Metabolism”

National Lipid Association Annual Scientific Sessions
Friday May 31, 2013

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Nuclear Receptors As Drug Targets for Treatment of Metabolic Diseases

- **PPARα**: Fibrates, Fish Oil
- **PPARγ**: TZDs
- **SREBP-2**: Statins, Ezetimibe, BAS
- **SREBP-1c**: Fish Oil, Insulin, Metformin
Sterol Response Element Binding Proteins (SREBPs)

Eberle D et al., 2004, Biochimie, 86: 839
SREBPs Regulate Cholesterol and Triglyceride

From: JD Horton, JL Goldstein, MS Brown. JCI 109:1125, 2002
SREBPs Are Regulated by Intramembrane Proteolysis (RIP)

Ligand Activated nuclear receptors

- TZDs
- PPARγ
- Fibrates (PPARα)
- hydroxysterols (LXRα)
- Statins
- Insulin
- SREBPs

RIP

Glucose Transport
Fatty acid oxidation
Reverse Cholesterol Transport
Cholesterol Uptake
SREBP’s What Do We Know?

- **SREBP-2**: Regulates Cholesterol Synthesis and LDL Uptake, tightly regulated by membrane sterols by process of regulated intramembrane proteolysis (RIP).

- **SREBP-1c**: Regulates Fatty Acid and Triglyceride Synthesis, Responsive to changes in feeding status via regulation by insulin, glucagon, and PUFA.

- **SREBP-1a**: Highly expressed in tumor cell lines, low level expression in normal cells, not responsive to dietary conditions. Can drive both cholesterol uptake and fatty acid synthesis.

**Question**: How did we arrive at this point?
Nuclear Protein That Binds Sterol Regulatory Element of Low Density Lipoprotein Receptor Promoter

- LDL receptor provides cholesterol to cells by binding and internalizing LDL-C
- Observation that LDL-Receptor gene responds to cholesterol availability
- Identified a sterol-responsive stretch of 10 bp in the LDL promoter, designated as sterol-regulatory-element-1.
- Identified a nuclear protein whose binding to the SRE-1 correlates with transcriptional activity.
- Protein designated as sterol regulatory element-1 binding protein (SREBP).

Single point mutation analysis of Repeat 2 of LDL receptor promoter
In transfected CV-1 cells

Michael R Briggs, Chieko Yokoyama, Xiaodong Wang, Michael S. Brown, and Joseph L. Goldstein
Nuclear Protein That Binds Sterol Regulatory Element of Low Density Lipoprotein Receptor Promoter: II Purification and Characterization

- Protein purified from nuclear extracts of HeLa cells (500 liters)
- Purified using DNA affinity Columns (38,000 fold purification)
- Dnase I footprinting analysis showed the protein bound to the SRE-1 sequence of the LDL receptor promoter region.
- Yielded protein bands between 59-68 kDa.

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**Table 1: Purification of SREBP from human HeLa cells**

<table>
<thead>
<tr>
<th>Step</th>
<th>Fraction</th>
<th>Protein* (mg)</th>
<th>Specific activity* (units/mg)</th>
<th>Total activity (units)</th>
<th>Purification fold</th>
<th>Recovery %</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Nuclear extract</td>
<td>6.009</td>
<td>230</td>
<td>144,540</td>
<td>1</td>
<td>100</td>
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<tr>
<td>2</td>
<td>SP-Sepharose</td>
<td>2.900</td>
<td>212</td>
<td>94,540</td>
<td>0.82</td>
<td>82</td>
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<tr>
<td>3</td>
<td>Ammonium sulfate</td>
<td>1.622</td>
<td>212</td>
<td>35,060</td>
<td>0.62</td>
<td>62</td>
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<tr>
<td>4</td>
<td>Superdex 300</td>
<td>4.98</td>
<td>230</td>
<td>114,540</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>Mutant Repeat 2 DNA-affinity (Column A)</td>
<td>445</td>
<td>212</td>
<td>94,540</td>
<td>0.82</td>
<td>82</td>
</tr>
<tr>
<td>6</td>
<td>Specific DNA-affinity (Column B)</td>
<td>1.7</td>
<td>50,405</td>
<td>65,400</td>
<td>1</td>
<td>100</td>
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<tr>
<td>7</td>
<td>Mutant Repeat 2 DNA-affinity (Column A)</td>
<td>0.97</td>
<td>70,230</td>
<td>68,123</td>
<td>0.95</td>
<td>95</td>
</tr>
<tr>
<td>8</td>
<td>Specific DNA-affinity (Column C)</td>
<td>0.004</td>
<td>8,784,000</td>
<td>35,136</td>
<td>38,194</td>
<td>31</td>
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</table>

* Protein concentration of the various fractions was determined as described under “Experimental Procedures.”
* One unit of activity is defined as described under “Experimental Procedures.”

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SREBP-1, a basic helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene


- Tryptic digestion of purified SREBP from HeLa cell nuclei
- Oligonucleotide primers used to prime PCR reactions
- Yielded 3 overlapping partial cDNAs = SREBP-1a
- pCY22 sequence lacks acidic amino acids in 1a = SREBP1c
- Peptide 4 used to identify SREBP-2 (next slide)
SREBP-2, a second basic-helix-loop-helix-leucine zipper protein that stimulates transcription by binding to a sterol regulatory element

Comparison of the amino acid sequences and domain structures of human SREBP-2 and SREBP-1a

DNA binding of recombinant SREBP-2 bHLH-ZIP domain to LDL receptor Promoter SRE-1 sequence.

Tissue distribution of mRNA and Expression of SREBP-2

Sterol-Mediated Proteolytic Release of SREBP mediates statin action

- Statins deplete sterols in hepatocyte membranes by inhibiting cholesterol synthesis.
- Lack of sterol binding releases SCAP/SREBP2 for transfer to Golgi.
- Proteolytic Cleavage of SREBP in Golgi releases transcriptionally active n-terminal fragment.
- nSREBP-2 activates LDL promoter to increase LDL receptor density on surface of liver.

JD Horton (pictured), JL Goldstein, MS Brown. JCI 109:1125, 2002
Role of SREBP-1c in Dyslipidemia in Insulin Resistance Associated with Obesity and T2DM

- Hyperinsulinemia
- Hypertension
- Hyperglycemia
- Dyslipidemia
  - Decreased HDL
  - Increased TG
  - Small dense LDL
- Genetics
- Altered fibrinolysis
- Endothelial dysfunction
- Aging
- Obesity
- Sedentary lifestyle

TG = triglycerides

Reusch JEB. *Am J Cardiol.* 2002;90(suppl):19G-26G.
Glucose and Lipid Metabolism are Dysregulated in Obesity and Type II Diabetes

Skeletal Muscle (Glucose Uptake) +

Insulin X

Liver

Lipid Synthesis Gluconeogenesis

Adipose Tissue (Lipolysis)

+ X

FFA

Glucose

Triglyceride (VLDL)
Defining the Role of SREBP-1c in the Pathogenesis of Dyslipidemia in Obesity and T2DM

- Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis related genes. Marc Foretz and Fabienne Foufelle, PNAS 96:12737, 1999.


- Our goal: to define the insulin response element (IRE) of the rat SREBP-1c promoter region and determine the molecular mechanisms underlying induction of SREBP-1c by insulin in Obesity and T2DM.
Regulation of the Rat SREBP-1c Promoter in Primary Rat Hepatocytes

Insulin activates the SREBP-1c promoter through the combinatorial actions of SREBP, LXR, Sp1 and NF-Y cis-acting elements.

Insulin activates the SREBP-1c promoter through the combinatorial actions of SREBP, LXR, Sp1 and NF-Y

Posttranslational processing of SREBP-1 in rat hepatocytes is regulated by insulin and cAMP

- Question: Does Insulin promote the ER to Golgi Transport of SREBP-1c and its proteolytic processing?

- Challenges:
  - low abundance of endogenous SREBP-1c
  - distinguishing transcriptional versus translational effects of insulin.

- Strategy: constitutive adenoviral mediated over-expression of His-tagged nascent SREBP’s

Eberle D et al., 2004, Biochimie, 86: 839

Experimental Approach

Ad-His-SREBP-1c-FLAG

Precursor SREBP-1c

Nuclear SREBP-1c

Insulin

Transcription factor

Regulatory domain

NH2

His

1

His

472

1129

COOH

FLAG
Insulin selectively promotes proteolytic processing of Ad- His-SREBP-1c but not Ad- his-SREBP-2

- SREBP-1c and 2 expressed in primary hepatocyte cultures using adenovirus
- Full length (117 kDa) precursor and active nuclear form (54 kDa) isolated from microsomal membrane fraction and nuclear fractions respectively
- Insulin increased processing of SREBP-1c but not SREBP-2

Insulin enhances post-translational processing of nascent SREBP-1c by promoting its phosphorylation and association with COPII vesicles

- Insulin exerts its actions via activating a series of kinases resulting in phosphorylation of key regulatory proteins
- Question: does insulin phosphorylate SREBP-1c?
- If so, is phosphorylation a regulatory step for posttranslational processing of SREBP-1c?

Phosphorylation of SREBP-1c Regulates its ER to Golgi Transport and Transcriptional Activity

- **Hypothesis**: phosphorylation of SREBP-1c mediates the effect of insulin and other effectors on SREBP-1c

- **Strategy**: expression of recombinant tagged SREBP-1c in hepatocytes, direct identification of phosphorylated sites by mass spectrometry

- **Alternate strategy**: identification of candidate phosphorylation sites by consensus sequence search

- **Evaluate candidate sites by site-directed mutagenesis**:
  - Serine to Alanine (S/A) = inactive
  - Serine to Aspartic Acid (S/D) = constitutively active

Qingming Dong
High level expression of tagged SREBP-1c in MCA Hepatoma Cells using Adenovirus

Recombinant expressed SREBP-1c undergoes proteolytic processing and nuclear localization in response to insulin treatment.

Purified nascent full-length SREBP-1c precursor (pSREBP-1c) isolated for mass spectroscopy using immunoprecipitation.
Identification of S73 and S92 as phosphorylated serines in SREBP-1c isolated from MCA hepatoma cells by mass spectroscopy.
### “Known” MAPK Sites on SREBP-1a and 1c

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Identified by:</th>
<th>Isoform (species)</th>
<th>*Rat SREBP-1c</th>
<th>Sequence (rat)</th>
<th>Putative Kinase(s)</th>
<th>Putative Functional Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>S63</td>
<td>SS (1)</td>
<td>1a (human)</td>
<td>S39</td>
<td>TGDTGPSSPGASSPE</td>
<td>p38 MAPK</td>
<td>enhanced transactivation (nuclear form)</td>
</tr>
<tr>
<td>S97</td>
<td>MS (1)</td>
<td>1a (rat)</td>
<td>S73</td>
<td>KVTPAPLSPPPSAPT</td>
<td>p38 MAPK/ERK</td>
<td>unknown</td>
</tr>
<tr>
<td>S73</td>
<td>MS (1)</td>
<td>1c (rat)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S117</td>
<td>SS(4)</td>
<td>1a (human)</td>
<td>S92</td>
<td>YPSVPPFSPGPGIKE</td>
<td>MAPK/ERK/JNK</td>
<td>enhanced transactivation (nuclear form)</td>
</tr>
<tr>
<td>S89</td>
<td>MS(1)</td>
<td>1c (rat)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T426</td>
<td>SS(3)</td>
<td>1a (human)</td>
<td>T395</td>
<td>PEVVETLTPPPSDAG</td>
<td>p38 MAPK (GSK)</td>
<td>enhanced transactivation (increased degradation)</td>
</tr>
</tbody>
</table>

Phosphomimetic (S to D) mutation of the putative MAPK phosphorylation site Ser39 enhances proteolytic processing.

- Phosphomimetic (S to D) Mutation of the identified MAPK site S39 enhanced ER to Golgi Transport of full-length precursor (p) SREBP-1c resulting in increased amount of transcriptionally active nuclear (n) SREBP-1c.

- Phosphomimetic mutation of S73 paradoxically reduced levels of pSREBP-1c.
Preventing Phosphorylation of SREBP-1a by MAP-Kinases Protects Mice from Fatty Liver and Visceral Obesity

- Created transgenic mice that over-expressed Wild-Type SREBP-1a and SREBP-1a with inactivating mutations of 3 MAPK sites
- Transgenic mice expressing WT SREBP-1a exhibited massively enlarged, fatty livers, hypertriglyceridemia, and massive accumulation of adipose tissue.
- Inactivating MAPK sites markedly attenuated effect of SREBP-1a over-expression.

Hypothesis: Altered Insulin signaling in obesity and T2DM leads to persistent activation of SREBP-1c

"Phosphorylation of SREBP-1 appears to be a regulatory step linking stress signals with metabolic events" (Kotzka et al.)
Identification of a gene variant in the master regulator of lipid metabolism SREBP-1 in a family with a novel form of severe combined hypolipidemia

- Investigated 190 unrelated German subjects including 69 subjects with very low LDL-C (<55 mg/dl) for genetic variations of the SREBF-1 or -2 genes.

- No sequence variation in the SREBPF-2 gene detected

- One sequence variation in exon 2 of the SREBF-1 gene common to both isoforms (c.332, C>T)

- Results in an exchange of proline to leucine at aa 111 of SREBP-1a and presumably SREBP-1c.

- Mutant SREBP-1a is not phosphorylated and is transcriptionally impaired.

- Mutation associated with markedly decreased plasma triglyceride and cholesterol as well as apoB

SREBPs: Master Regulators of Cholesterol and Triglyceride Metabolism

- SREBPs play a pivotal role in the pathogenesis of a wide range of metabolic disorders including hyperlipidemia, T2DM, obesity, hepatic steatosis, and cancer.

- SREBPs mediate the effects of a wide range of pharmacologic agents used in treatment of metabolic diseases.

- Understanding of the molecular mechanisms underlying regulation of SREBPs is essential to understanding the pathogenesis of many metabolic diseases.

- SREBPs provide a novel treatment target for metabolic complications of obesity and T2DM.
AMPK Phosphorylates and Inhibits SREBP Activity to Attenuate Hepatic Steatosis and Atherosclerosis in Diet-Induced Insulin-Resistant Mice

A Synthetic Polyphenol S17834 Stimulates AMPK Activity and protects against Hepatic Steatosis in High-Fat, High Sucrose Diet - fed LDL-R Deficient Mice.

Sterol Regulatory Element Binding Protein 1a Regulates Hepatic Fatty Acid Partitioning by Activating Acetyl Coenzyme A Carboxylase 2

- Created a SREBP-1a knockout mouse by insertional mutagenesis
- Normal regulation of lipogenic genes (FAS, ACC1)
- Microarray analysis: Reduced expression of ACC2
- Increased fatty acid oxidation during fasting

Question: Is induction of SREBP-1c and lipogenic enzymes in rats pertinent to obese humans?

Liver biopsy samples from Morbidly Obese (MO) humans undergoing bariatric surgery and those undergoing abdominal wall revision following massive weight loss (MWL).

Following weight loss observed reduced hepatic expression of SREBP-1c, fatty acid synthase (FAS) and Acetyl-CoA-carboxylase (ACC).

Conclusion: SREBP-1c mediated enhanced de novo lipogenesis plays a role in hyperlipidemia in obese human.

Marshall B Elam, George S. Cowan (pictured) et al Metabolism 59:587, 2010
Dietary Olive Oil and Menhaden Oil Mitigate Induction of Lipogenesis in Hyperinsulinemic Corpulent JCR:LA-cp Rats

- Question: Does Dietary PUFA reduce SREBP-1c?

- Obese (OB) JCR Rats (leptin receptor defect) fed rat chow (c) olive oil (O) or fish oil (F).

- SREBP-1c and Lipogenic enzymes increased in OB

- PUFA (Fish Oil) effectively reduced expression of SREBP-1c and its target lipogenic enzymes (FAS, ACC1)

- Forms the basis of hypotriglyceridemic effect of fish oil (EPA, DHA)

Xiong Deng, Marshall B. Elam et al Endocrinology 145:5847, 2004