Diagnosis of Type 2 diabetes susceptibility genes: Synthesis and Use of a Novel O-GlcNAcase-specific Inhibitor and Fluorogenic Substrate

[An O-GlcNAcases-Specific Inhibitor and Fluorogenic Substrate Engineered by the Extension of the N-Acetyl Moiety]

Description of Technology:

This technology relates to the synthesis and evaluation of a selective inhibitor of O-linked GlcNAcase, an enzyme that removes N-acetylglucosamine from nuclear and cytoplasmic proteins, and is encoded by one of the type 2 diabetes susceptibility genes (MGEA5). Specifically, a novel analogue of O-(2-acet-amido-2-deoxy-D-glucopyranosyline) amino-N-phenylcarbamate (PUGNAc) has been synthesized. It is known that PUGNAc alters O-GlcNAc modifications of proteins within the insulin-signaling cascade and induces insulin resistance in fat cells. The analogue to PUGNAc provides enhanced specificity for O-GlcNacase and may be used to test for the presence of the diabetes susceptibility gene in human tissue or blood samples. A similar modification to produce a fluorogenic substrate confers selective recognition of O-GlcNAcase.

Applications:

Recent evidence suggests that deregulation of cellular O-GlcNAc levels might play a role in Type 2 diabetes, cancer and neurological disorders. The O-GlcNAcase is involved in the post-translational deglycosylation of a large number of nucleic and cytoplasmic proteins, such as transcription factors, nuclear pore proteins, and enzymes, including those involved in the etiology of Type 2 diabetes. Thus this analogue to PUGNAc may be used in the diagnosis of Type 2 diabetes.

Advantages:

- The PUGNAc analogue reported here is a selective inhibitor of O-GlcNAcase that does not alter the activity of hexosaminidase A and hexosaminidase B.
- The pentanamide fluorogenic substrate is solely utilized by O-GlcNAcase and is not a substrate for hexosaminidase A or B.
- This fluorogenic substrate is suitable for high-throughput screening (HTS) of the O-GlcNAcase.
- These reagents provide the first means for monitoring GlcNAcase activity independent of the related enzymes hexosaminidase A and hexosaminidase B.
- These components offer greater specificity than pervious reagents used to monitor the enzymes.

Development Status:

- Analogues of O-(2-acet-amido-2-deoxy-D-glucopyranosyline)amino-N-phenylcarbamate (PUGNAc), as well as the pentanamide fluorogenic substrate, have been synthesized and are available for licensing for the development of a diagnostic or therapeutic agent.

Inventors:

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Publications:

Kim EJ, Perreira M, Thomas CJ, Hanover JA. *An O-GlcNAcase-specific inhibitor and substrate engineered by the extension of the N-acetyl moiety*.J Am Chem Soc. 2006 Apr 5;128(13):4234-5. No abstract available.

Kim EJ, Kang DO, Love DC, Hanover JA. *Enzymatic characterization of O-GlcNAcase isoforms using a fluorogenic GlcNAc substrate.* Carbohydr Res. 2006 Jun 12;341(8):971-82. Epub 2006 Apr 11. [Pubmed reference]

Patent Status:

DHHS Reference No. E-229-2006**,** a provisional application has been filed.

Licensing Status:

Available for exclusive or non-exclusive licensing

Licensing Contact:

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Collaborative Research Opportunity:

The National Institute of Diabetes and Digestive and Kidney Diseases, Laboratory of Cell Biochemistry and Biology is seeking statements of parties interested in collaborative research to further develop, evaluate, or commercialize the use of PUGNAc analogs as diagnostic or therapeutic agents. Please contact John A. Hanover, at jah@helix.nih.gov or Rochelle S. Blaustein at Rochelle.Blaustein@nih.gov for more information.

Research Focus and Selected Publications for Principal Investigator

Dr. John A. Hanover, Chief Laboratory of Biochemistry and Biology

Dr. Hanover's laboratory focuses on (1) the mechanism of nuclear transport and (2) the molecular features of a novel, glycan-dependent, signal transduction cascade. The nuclear transport of transcription factors, nuclear kinases, steroid hormone receptors, and replication factors often serves a critical regulatory function. His laboratory is examining the mechanisms of nuclear import, export, and subnuclear targeting. They have identified a novel nuclear transport pathway involving calmodulin. This pathway has been shown to play a role in mammalian sex determination and stem cell differentiation. Dr. Hanover's group is also identifying additional components of this pathway using yeast genetics and chemical biology approaches. The nuclear pore complex (NPC) mediates the transport of mRNA and proteins across the nuclear envelope.

Many components of the nuclear pore are modified by a novel modification: O-linked N-acetylglucosamine (O-linked GlcNAc). The modification also occurs on transcription factors and certain oncogenes and tumor suppressors. Current evidence suggests that the O-linked GlcNAc transferase mediates a novel glycan-dependent signal transduction pathway. The laboratory has molecularly cloned and characterized the human O-linked GlcNAc transferase responsible for glycosylating nuclear pore proteins. This enzyme is expressed as differentially targeted isoforms in man and is localized to both the nucleus and the mitochondria. When expressed in E. coli, the human O-linked GlcNAc transferase is catalytically active. The lab has recently solved the X-Ray structure of the substrate recognition domain of OGT and is beginning to understand how it recognizes its many intracellular targets. Although the enzyme is found in a number of target tissues, it is most highly expressed in human pancreatic beta cells, consistent with a role in glucose-sensing. Based on its substrate specificity and molecular features, Dr. Hanover has proposed that O-linked GlcNAc transferase is the terminal step in a glucose-responsive pathway that becomes disregulated in diabetes mellitus (NIDDM). The enzyme catalyzing O-GlcNAc removal, O-GlcNAcase, has also been identified, expressed and shown to exist as differentially targeted isoforms in man. The laboratory is also using the genetically amenable C. elegans model to examine the physiological impact of the enzymes of O-GlcNAc cycling. Through the use of reverse genetics, knockout, and other transgenic models, the role of these essential genes in signal transduction and pathogenesis of diabetes mellitus is being exploring

Publications

1. Zhao Y, Conze DB, Hanover JA, Ashwell JD Tumor necrosis factor receptor 2 (TNFR2) signaling induces selective c-IAP1-dependent ASK1 ubiquitination and terminates MAP kinase signaling. *J Biol Chem* , 2007. [Full Text/Abstract]

2. Ying H, Furuya F, Zhao L, Araki O, West BL, Hanover JA, Willingham MC, Cheng SY Aberrant accumulation of PTTG1 induced by a mutated thyroid hormone beta receptor inhibits mitotic progression. *J Clin Invest* (116): 2972-2984, 2006. [Full Text/Abstract]

3. Furuya F, Hanover JA, Cheng SY Activation of phosphatidylinositol 3-kinase signaling by a mutant thyroid hormone beta receptor. *Proc Natl Acad Sci U S A* (103): 1780-5, 2006. [Full Text/Abstract]

4. Kim EJ, Perreira M, Thomas CJ, Hanover JA An O-GlcNAcase-Specific Inhibitor and Substrate Engineered by the Extension of the N-Acetyl Moiety. *J Am Chem Soc* (128): 4234-5, 2006. [Full Text/Abstract]

5. Forsythe ME, Love DC, Lazarus BD, Kim EJ, Prinz WA, Ashwell G, Krause MW, Hanover JA Caenorhabditis elegans ortholog of a diabetes susceptibility locus: oga-1 (O-GlcNAcase) knockout impacts O-GlcNAc cycling, metabolism, and dauer. *Proc Natl Acad Sci U S A* (103): 11952-7, 2006. [Full Text/Abstract]

6. Kim EJ, Kang DO, Love DC, Hanover JA Enzymatic characterization of O-GlcNAcase isoforms using a fluorogenic GlcNAc substrate. *Carbohydr Res*, 2006. [Full Text/Abstract]

7. Kim CS, Furuya F, Ying H, Kato Y, Hanover JA, Cheng SY Gelsolin: a novel thyroid hormone receptor {beta} interacting protein that modulates tumor progression in a mouse model of follicular thyroid cancer. *Endocrinology* , 2006. [Full Text/Abstract]

8. Perreira M, Kim EJ, Thomas CJ, Hanover JA Inhibition of O-GlcNAcase by PUGNAc is dependent upon the oxime stereochemistry. *Bioorg Med Chem* (14): 837-46, 2006. [Full Text/Abstract]

9. Lazarus BD, Love DC, Hanover JA Recombinant O-GlcNAc transferase isoforms: identification of O-GlcNAcase, yes tyrosine kinase, and tau as isoform-specific substrates. *Glycobiology* (16): 415-21, 2006. [Full Text/Abstract]