Identification of Genes Induced by Growth Hormone in Rat Liver Using cDNA Arrays

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ABSTRACT

Growth hormone (GH) is a pleiotropic cytokine that acts upon its target cells to regulate their growth, differentiation and metabolism. GH is thought to act by altering gene expression in target cells, but few GH-regulated genes are known. In this study, we used cDNA array analysis to identify genes rapidly induced in the liver of GH-deficient dwarf rats following a single systemic injection of GH. Eight genes were found to upregulate their mRNA expression within 1-3 hours of GH administration, results which were confirmed by northern analysis. The identity of these genes suggests GH may influence a diversity of cellular processes. A role for GH in regulating cytokine and growth factor signalling is suggested by upregulation of mRNAs encoding three signal transducers: a subunit of the receptor for IL-6-type cytokines (gp130), STAT3 (signal transducer and activator of transcription) and p38MAPK (mitogen activated protein kinase). Two genes involved in DNA repair and cell cycle control, APEN (apurinic endonuclease) and GADD45 (growth arrest and DNA damage 45) were upregulated. Other induced genes include those encoding a lactate transporter (MCT-1), an extracellular matrix remodelling enzyme, MT1-MMP (membrane type 1 matrix metalloproteinase) and an acute phase protein (fibrinogen beta). In summary, this work is the first to apply cDNA arrays to the study of peptide hormone action in vivo and has identified 8 novel GH target genes.

Following birth, the progression of normal human growth is driven by the action of growth hormone (GH). GH acts upon a variety of tissues by activating a specific cell surface receptor, initiating several intracellular signalling pathways that direct changes in gene expression. Regulation of gene expression in this manner is thought to underlie many of the effects of GH on cellular metabolism, proliferation and differentiation.

While there is considerable knowledge of the biochemical events involved in GH receptor activation and intracellular signal transduction (reviewed in (1)), relatively few genes are known to be directly regulated by GH. Most of the GH-regulated genes known have been identified from studies of liver tissue from rats deficient in GH (i.e.: hypophysectomised). These studies have shown that GH administration rapidly induces mRNA encoding insulin-like growth factor 1 (IGF-1), IGF binding protein (IGFBP)-3, the acidic labile subunit (ALS) of the IGF binding complex, fatty acid binding protein (FABP), serine protease inhibitor (spi) 2.1, epidermal growth factor receptor (EGFR), suppressors of cytokine signalling (SOCS) genes, as well as the transcription factors c-fos, c-jun, interferon response factor (IRF)-1 and hepatocyte nuclear factor (HNF)-6 (2-10). GH is also known to repress expression of IGFBP-1 (8). In addition, expression of certain male and female-specific liver genes, for example several cytochromes P450, has been shown to be dependent on the sex-specific pattern of GH secretion (11-13).

Further advances in our knowledge of how GH regulates gene expression and how this directs the physiological actions of GH requires that more GH target genes be identified. To this end, we have used cDNA array analysis to detect genes whose mRNA expression changes in liver tissue of GH-deficient dwarf rats following a single systemic injection of GH. Northern blot analysis confirmed upregulation of 8 previously unreported transcripts by GH. The identity of these genes provides new insight into the understanding of GH action.

Materials and Methods

Animals and Treatments

All experiments were carried out under University of Queensland ethical guidelines. GH-deficient Lewis dwarf rats (dw/dw) were housed under controlled conditions with food and water ad libitum. Animals were studied at 7-9 weeks of age. Male litter mates were administered with either 400μl of vehicle (50mM NaHCO3) or 200μg of bGH (Cynamid, Princeton, NJ, USA) dissolved in 400μl of vehicle by a single intraperitoneal injection. At defined times following injection, rats were killed by spinal dislocation and portions of the left liver lobe were quickly removed and processed immediately.
RNA isolation and Northern blotting

Total RNA was isolated from liver tissue using TRIzol reagent (Life Technologies, Mulgrave, Australia). Poly (A)+ RNA was purified from total RNA using oligo-dT conjugated latex beads (Qiagen, Clifton Hill, Australia). For northern, total RNA (15μg) was electrophoresed on a denaturing 1% agarose gel and transferred to a nylon membrane (Micron Separations, Westborough, MA, USA) and UV-crosslinked. Membranes were hybridised with 32P-labelled cDNA probes in UltraHyb hybridisation solution (Ambion, TX, USA) and washed according to the manufacturer's protocol. Results were visualised by autoradiography. Probes were synthesised from specific cDNAs using the Rediprime II kit (Amersham, UK) with [γ-32P]ATP (Geneworks, Adelaide, Australia). cDNA clones used to generate probes were obtained from the Research Genetics library (Huntsville, Alabama, USA).

cDNA array analysis

'Atlas' nylon cDNA arrays, consisting of 588 rat DNA fragments generated from known cDNAs by specific PCR amplification, were purchased from Clontech (Palo Alto, CA, USA). cDNA probes for Atlas array hybridisation were generated according to the Clontech Atlas protocol from 1μg of poly(A)+ RNA by reverse transcription (SuperscriptII RT, Gibco). First-strand synthesis incorporated [γ-32P]ATP (Geneworks) and was primed by addition of a mixture of primers specific to each of the genes present on the Atlas array (primer mix supplied by Clontech). First-strand cDNA probes were subsequently purified from unincorporated label by size-fractionation through a ChromaSpin-200 gel matrix column (Clontech). Hybridisation was performed according to the Clontech Atlas protocol. Results were analysed using a BioRad phosphorimager and analysis software. Array data were normalised according to the average signal intensity across the entire membrane. All procedures were performed in parallel for control and GH-treated samples. cDNA clones corresponding to those DNA molecules grided on the Atlas array were identified using the Unigene, dbEST and Clontech Atlas databases.

Results

The effect of a single dose of bGH (200μg), administered by intraperitoneal injection, upon mRNA levels in the dwarf rat liver was examined using Atlas cDNA arrays representing 588 distinct rat genes. Liver tissue was harvested for poly(A)+ RNA preparation at 1 and 3hr after GH administration. GH induction of IGF-1 and CIS mRNAs in all samples was checked by northern analysis prior to use. Radiolabelled cDNA probes synthesised from each sample of poly(A)+ RNA were hybridised to Atlas cDNA arrays and the resulting hybridisation patterns were compared between control and GH treated animals.

We observed that most array hybridisation signals did not change significantly upon GH treatment. Genes that did alter their hybridisation signals

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<th>Time (h) after GH administration</th>
<th>Fibrinogen β</th>
<th>gp130</th>
<th>STAT3</th>
<th>p38MAPK</th>
<th>GADD45</th>
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FIG 1. Northern blot analysis of genes identified to be GH-induced using cDNA array hybridisation. 15μg of total RNA isolated from dwarf rat liver treated with GH for the times shown was blotted and hybridised to probes for fibrinogen β, glycopenitin (gp) 130, signal transducer and activator of transcription 3 (STAT3), p38 mitogen activated protein kinase (p38MAPK), growth arrest and DNA damage inducible gene 45 (GADD45), apurinic endonuclease (APEN), membrane type-1 matrix metallopeptidase (MT1-MMP) and monocarboxylate transporter 1 (MCT1). 18S was used to control for loading variations. All genes progressively increased mRNA levels during GH treatment, except GADD45 which was transiently induced.
included four genes that have previously been identified as GH-regulated (FABP, EGF R, IGFBP3 and IGFBP1). A further 20 genes, whose hybridisation signals altered by a factor of greater than 50% at either 1 or 3hr after GH administration, were selected for northern analysis. mRNAs encoding 8 of these genes were confirmed to be reproducibly GH-induced in northerns (figure 1).

Discussion

This work has identified 8 previously unreported target genes for GH in rat liver: fibrinogen β, glycoprotein (gp) 130, signal transducer and activator of transcription 3 (STAT3), mitogen activated protein kinase p38 (p38MAPK), growth arrest and DNA damage inducible protein 45 (GADD45), apurinic endonuclease (APEN), membrane-type 1 matrix metalloproteinase (MT1-MMP) and monocyte transporter 1 (MCT1).

Fibrinogen β mRNA encodes one component of the blood clotting protein fibrinogen. Fibrinogen is rapidly induced by cytokines during the acute phase response to infection or tissue injury, along with other acute phase proteins (14). GH has been reported to induce synthesis of acute phase proteins, including fibrinogen, in healthy animals (15). However, during the acute phase response, GH treatment attenuates acute phase protein synthesis (16) - indicative of complex crosstalk between GH and acute phase cytokine signalling (17).

gp130, STAT3 and p38 MAPK are all cytokine and/or growth factor signal transducers. gp130 is the common receptor subunit of IL-6-type cytokines (18). STAT3 is a transcription factor activated by gp130 and other cytokine receptors, including the GH receptor (19). p38 MAPK transduces signals of numerous cytokines and growth factors (20). These signalling agents are critically involved in control of cell growth and differentiation and cellular responses to infection and injury. GH-regulation of mRNA encoding these signal transducers may be a means by which GH can regulate cytokine and growth factor responses; in the liver, these might include modulation of hepatic growth, metabolism or the acute phase response.

GADD45 and APEN have important functions in control of DNA damage and in the response of cells to stress (21, 22). In these roles, GADD45 acts to inhibit mitotic growth (23) and APEN is both a DNA repair enzyme (24) and an activator of several transcription factors (25, 26). GH-upregulation of GADD45 and APEN mRNA suggests GH may have a role in maintaining defences against cell stress and DNA damage.

MCT1 encodes a member of a family of plasma membrane lactate transporters (27). This transporter facilitates lactate uptake by a variety of tissues, including liver. Low levels of MCT1 expression in humans, caused by heterozygous missense mutations in the MCT1 gene, lead to decreased lactate uptake and symptoms of muscle injury on exertion (28). GH induction of MCT1 mRNA may boost lactate uptake by the liver, an effect which would complement the ability of GH to stimulate hepatic gluconeogenesis (29) by providing more lactate for production of glucose.

MT1-MMP is a transmembrane protein whose extracellular domain has metalloproteinase activity capable of degrading extracellular matrix (ECM) proteins (30). ECM breakdown by matrix metalloproteinases is an integral part of tissue development, morphogenesis and remodelling as well as tumor metastasis (30). MT1-MMP plays particular roles in postnatal growth, skeletal development and angiogenesis (31, 32). Activation of MT1-MMP mRNA expression suggests a mechanism by which GH could regulate matrix remodelling during cellular growth and differentiation.

In summary, the 8 novel GH target genes described here suggest potential new mechanisms for GH modulation of liver acute phase responses, cellular growth and differentiation, DNA repair and glucose metabolism. It is readily conceivable that regulation of these genes may also contribute to the effects of GH in other target tissues. In addition, these genes provide endpoints for analysis of the mechanisms of transcriptional regulation by GH.

Acknowledgement

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References


