Background

The completion of human and rodent genome sequences [1-3] has brought the post-genomic era to the field of endocrine research. Detailed genetic maps of the main endocrine models can now be used to study the molecular basis of endocrine disease and the molecular mechanisms of hormone actions. The possibility to explore expression data of thousands of genes across multiple experimental paradigms promise to rapidly increase our understanding of biological systems [4,5]. The acquisition of experimental data at a genomic scale requires high throughput technologies such as DNA microarray analysis. Microarrays enable the simultaneous assessment of expression levels of tens of thousands of gene products in an ease to perform assay. Microarrays are especially attractive to the field of endocrine research because regulation of gene expression is an important mechanism whereby hormones exert their physiological actions. This is obvious in the case of steroid and thyroid hormones, which use intracellular receptors belonging to the nuclear receptor family of transcription factors [6]. Peptide hormones also regulate gene expression after activating complex cascades of intracellular signaling events upon binding to transmembrane receptors [7]. If the relation between hormones and the expression of different genes could be annotated, the abundant knowledge concerning endocrine physiology might be used to clarify the biological function of those genes. On the other hand, because expression profiles are rich in information, they are suitable to study the complex and pleitropic actions of hormones.

Here we analyzed a compilation of rat liver expression profiles from experiments designed to study gender and hormone actions in order to provide novel insight into the mechanisms of action of specific hormones. The dataset used in this study comprises the actions of thyroid hormone (T3), 17-α-ethinylestradiol and GH in liver. The data is freely available from the Endocrinology Gene Expression Database – http://www.cmm.ki.se/EndoGED and have also been deposited in Gene Expression Omnibus http://www.ncbi.nlm.nih.gov/geo/. Using this collection of microarray data, we analyzed the differential contribution of estrogens and GH to the regulation of gender differentiated liver gene expression. We also compared the actions of GH and T3 in liver and found a small overlap comprising genes involved in lipogenesis suggesting the common regulation of SREBP transcription factors. The regulation of SREBP1 by GH and thyroid hormone was analyzed.

Results

Exploring hormonal regulation of gene expression in liver

Several expression profiles where compiled and used to obtain insight into the hormonal regulation of liver physiology. The following six experiments were analyzed together (Table 1): 17-α-ethinylestradiol treatment of male rats, infusion of bGH in young (3 month) male rats [8], infusion of hGH in old (2 years) male rats [9], bGH treatment of primary hepatocytes isolated from young male rats, comparison of female and male rats [8], and the rapid effects of T3 treatment of hypothyroid mice [10]. The following questions were formulated: Can expression profiling be used to clarify the physiological actions of hormones in the liver? Can promoter analysis of hormonally regulated genes provide novel insight into the mechanism of hormone actions? It should be noted that our intentions were not to exhaustively explore the data set but rather to illustrate the utility of microarray data mining in endocrine research. The experiments included in this analysis were not specifically designed to answer the questions formulated in the present study although they are sufficient to test our hypotheses. This mimics the situation when the experimental biologist try to derive knowledge from a set of disparate experiments performed in different laboratories, using different experimental designs and microarray technologies. Importantly, we have taken all possible measures to minimize systematic experimental errors. All the arrays used for analysis have been fabricated in house from a unique set of PCR products and these have been validated in numerous studies [7-15]. The protocols for labeling and data analysis were also similar along all the experiments. Within each of the experiments included in this analysis, we have accounted for biological variability by independent replication of the measurements using RNA from individual animals.

Unsupervised clustering algorithms were first used on the entire dataset to gain a global view of different hormone actions in liver. Average-linkage hierarchical clustering (using Euclidean distance as measurement of similarity) was used to evaluate the relation between the expression profiles. As shown in Figure 1a, the effects induced by GH treatment in young males were similar to those induced by GH treatment in old animals. This observation supports the robustness of the GH effects since the designs of the two experiments differ not only regarding the age of the animals but also in the dose and type of hormone administered (bovine versus human GH). A calculation of the correlation coefficients between bGH-induced expression changes and the rest of the experiment groups supports this conclusion (Figure 1b). Interestingly, the expression changes induced by GH treatment of primary rat hepatocytes cultured on matrigel show a positive, although small, correlation with the effects observed *in vivo* (Figure 1a and 1b). This suggests that hepatocytes significantly contribute to the expression changes measured in intact liver upon GH treatment. The fact that well-known GH-regulated genes: insulin-like growth factor 1(IGF-1) and CYP2C12 [16] are also regulated in hepatocytes helps to substantiate our conclusion. The similar