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Hypothesis

Functional Connections and Pathways of Coenzyme Q_{10}-inducible Genes: An In-silico Study

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Summary

Coenzyme Q\(_{10}\) (CoQ\(_{10}\), ubiquinone) is an essential cofactor in the electron transport chain, serves as a potent antioxidant in mitochondria and lipid membranes, and is often used as a dietary supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular mitochondria and lipid membranes, and is often used as a dietary supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases. Recently, we obtained evidence that CoQ\(_{10}\) (Kaneka supplement for a number of diseases including cardiovascular diseases.

INTRODUCTION

Coenzyme Q\(_{10}\) (CoQ\(_{10}\)) is an essential electron carrier and proton translocator in the mitochondrial respiratory chain (1). CoQ\(_{10}\) is also an obligatory cofactor of the dihydroorotate dehydrogenase (2) and serves as a potent antioxidant in membranes by directly scavenging radicals (3, 4) and regenerating \(\alpha\)-tocopherol (5–7). More recently, the role of CoQ\(_{10}\) in the function of uncoupling proteins was discussed (8–10). The functional diversity of CoQ\(_{10}\) reflects its suitability for applications in clinical studies as an dietary supplement for a number of diseases (11). These include Parkinson’s disease (1, 12–15), mitochondrial myopathies (16, 17), age-related macular degeneration (18), migraine (19), idiopathic asthenozoospermia (20, 21), and cardiovascular diseases (22–24). The molecular mechanisms by which CoQ\(_{10}\) mediates these beneficial effects are uncertain. We (25) and others (26, 27) obtained evidence that CoQ\(_{10}\) influences the expression of hundreds of genes involved in different cellular pathways. To decipher the functional and regulatory connections of these genes we employed bioinformatic techniques to access the actions of CoQ\(_{10}\) in detail. This in-silico approach revealed that CoQ\(_{10}\) modulates inflammatory pathways via gene expression. Thus, some of the effects of CoQ\(_{10}\) on vascular health may be due to this property.

MATERIAL AND METHODS

In-Silico Analyses

We used the freely-available part of Genomatix Software 2006 (www.genomatix.de). The CoQ\(_{10}\)-regulated genes were taken from our recent publication (25). In that study, we incubated intestinal Caco-2 cells with 50 \(\mu\)M CoQ\(_{10}\) of a liposomal preparation for 24 h. After exposition, gene array technology revealed changes in steady-state mRNA levels for hundred of human genes. The accession numbers of these CoQ\(_{10}\)-regulated genes (25) were uploaded to Bibliosphere-PathwayEdition (BSPE). This text mining tool identifies functional connections based on co-citations of gene names and synonyms (28). The co-citation filter ‘gene…function word…gene’ (GFG level B3) was applied. The accession numbers of filtered genes were then uploaded to
Gene2Promoter software which allowed the identification of promoter regions based on individual transcripts (29). The obtained promoter sequences were adjusted to 600 bp, 500 bp upstream and 100 bp downstream of transcriptional start sites, and deposited in MatInspector to identify functional and common modules in input promoters (30, 31). A common sites analysis was performed. We chose only models common to at least three input sequences (60%). The minimum and maximum distance between two elements was chosen 5 and 50 bp, respectively.

RESULTS
The Text-mining System BiblioSpherePathwayEdition (BSPE) Revealed 17 CoQ10-sensitive Genes which are Functionally Connected by Four Different Pathways
Recently, we identified 464 differentially regulated genes in the intestinal Caco-2 cell line after CoQ10-treatment at a threshold-factor of at least 2.0 in three independent experiments (25). These genes were used to identify their putative functional connections by using the text-mining system BSPE. Of 464 uploaded transcripts, 413 were recognized by the program. Transcripts which showed co-citations with transcripts containing transcription factors, functional co-citations (GGG level B3) and co-citations with other genes of the input list were selected. Based on these stringent criteria, we identified 19 CoQ10-inducible genes whereby 17 genes are functionally connected by signalling pathways of G-protein coupled receptors, JAK/STAT, integrin, and theta-receptor (Fig. 1, Table 1). Since five of these genes code for proteins involved in inflammation (IL5, thrombin, vitronectin, vitronectin receptor, C-reactive protein), a sub-analysis was performed. As shown in Fig. 2, these genes are connected by the transcription factor NFkB1.

The MatInspector-based Promoter Analysis of 17 Connected CoQ10-sensitive Genes Revealed Common Regulatory Modules in Three Inflammatory Genes
To identify putative functional and common frameworks in the regulatory regions of the 17 identified CoQ10-inducible genes, their promoter sequences were extracted from NCBI GenBank using Genomatix Gene2Promoter software and were deposited in MatInspector. We searched for common frameworks containing at least two transcription factor binding sites (TFBS) at a distance between 5 and 50 bp, and the quorum constraint was adjusted to 60%. The search was done with combinations of five promoter sequences. A framework common to all input promoters or common frameworks with five elements was not found. Whereas frameworks with two or three elements are common in input genes, we identified only one framework with four elements. As shown in Fig. 3, a common framework containing the binding sites of the transcription factor families EVI1 (ectropic virulent integration site 1 encoded factor), HOX (homeodomain transcription factor) and CLOX (cut-like homeo box) were found in the promoters of IL5, C-reactive protein, and vitronectin receptor.

DISCUSSION
In this study we have performed an in-silico approach to decipher the functional and regulatory connections of 464 human genes which were recently identified (23) as 'CoQ10-inducible'. To obtain convincing connections we combined a literature analysis with a transcriptional factor binding site search. A recent analysis of genes encoding small leucine rich proteoglycans showed indeed, that this combined analysis seems to be more predictive than sole searches for transcription factor binding sites (32, 33). Although the analysed CoQ10-inducible gene can be grouped according to for example mitochondrial respiration or plasma membrane redox component, our strategy with rigorous criteria revealed that 17 CoQ10 inducible genes are connected by four different cellular signalling pathways. Whereby, the genes of IL5, thrombin, vitronectin, vitronectin receptor, and C-reactive protein (CRP) seem to be regulated by NFkB1 and promoter frameworks containing the transcription factors EVI, HOX, and CLOX. Although the precise roles of these transcriptional factors are not completely unravelled, they are essentially involved in different aspects of development and are linked to several human diseases including inflammation (34–37). Accordingly, IL5, thrombin, vitronectin and its receptor as well as CRP are key components in similar steps of inflammation processes. Although the in-vivo relevance of these effects has to be clarified, an up-regulation of these genes could for example sensitize the inflammatory responses of monocytes.

Whereas IL5 is mainly responsible for the tissue damage observed in allergic disorders (38), the other identified genes seem to be important in the development of atherosclerosis. CRP has been reported as a potent peptide that causes platelet adhesion to epithelial cells, thereby regulating atherothrombosis (39). This step is also regulated by vitronectin and its receptor, since they interact with thrombin and antithrombin III (40). The finding that expression of vitronectin and its receptor is modulated by CoQ10 is particularly interesting for several reasons. First, plasma vitronectin levels are increased in patients with coronary atherosclerosis (41). Second, it has been shown that vitronectin-mediated cell survival also includes regulation of NFkB-activity (42). Third, vitronectin is essential for monocyte adhesion to endothelium (43). Finally, dose-, and time-dependent inhibitory properties of CoQ10 on platelet aggregability have been already shown in a previous study with swine (44). Another study indicated a significant inhibition of vitronectin-receptor expression in human
Figure 1. BiblioSphere Pathway view network of input genes regulated by CoQ10. A network of 17 genes was identified by analysis of 464 CoQ10-inducible genes with the BiblioSpherePathwayEdition software package based on co-citations with transcription factors, functional co-citations, and co-citations with other genes in the network. The abbreviations and descriptions are listed in Table 1. The genes DNTT and NP are not part of the network. IN, input gene; ST, gene product is part of a Genomatix signal transduction pathway; STKE, gene is part of a SignalTransductionKnowledgeEnviromental connection map.
Therefore, one possible mechanism by which CoQ10 produces positive effects in cardiovascular diseases is through platelet inhibition. This putative beneficial mechanism is accompanied by other effects of CoQ10 on cardiovascular diseases. This includes protection of LDL from oxidation, prevention of free-radical damage caused by neutrophils and reduction of oxidative injury by endothelial cells (11, 46, 47). Of course, based on our in-vitro data the effect of CoQ10 on vascular health in-vivo need to be studied in future animal and/or human intervention studies. In those studies, tissue specific effects have to be taken into account. Further, the applied CoQ10 concentration of 50 μM for 24 h in-vitro is difficult to achieve in humans. On the other side, the exposure time in-vivo is quite longer than in our in-vitro study. Again, in-vivo studies are necessary to evaluate the effect of CoQ10 on gene expression and vascular health. In addition, future analysis of the gene expression data on protein and metabolite level is necessary.

Taken together, based on our in-silico analysis of more than 400 CoQ10-inducible genes, we obtained evidence that a part of the CoQ10 regulation plays an important role in inflammatory response. Since these effects are based on in-vitro study, the effect of CoQ10 on vascular health in-vivo needs to be addressed in further animal and/or human intervention studies.

Table 1
CoQ10-inducible genes in the identified network (see Fig. 1)

<table>
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<th>Symbol</th>
<th>Transcripta</th>
<th>Description</th>
<th>Fold change by CoQ10b</th>
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<tr>
<td>SST</td>
<td>NM_001048</td>
<td>Somatostatin</td>
<td>+2.3</td>
</tr>
<tr>
<td>IL5</td>
<td>NM_000879</td>
<td>Interleukin 5, colony-stimulating factor, eosinophil</td>
<td>+5.6</td>
</tr>
<tr>
<td>F2</td>
<td>NM_000506</td>
<td>Coagulation factor II, thrombin</td>
<td>+2.4</td>
</tr>
<tr>
<td>RNASE2</td>
<td>NM_002934</td>
<td>Ribonuclease, RNase A family</td>
<td>+16.0</td>
</tr>
<tr>
<td>PYGM</td>
<td>NM_005609</td>
<td>Glycogen phosphorylase</td>
<td>+3.5</td>
</tr>
<tr>
<td>ITGAV</td>
<td>NM_002210</td>
<td>Integrin alpha V, vitronectin receptor</td>
<td>+3.0</td>
</tr>
<tr>
<td>DNTT</td>
<td>NM_004088</td>
<td>Deoxynucleotidyltransferase, terminal</td>
<td>+3.3</td>
</tr>
<tr>
<td>STAT6</td>
<td>NM_003153</td>
<td>Signal transducer and activator of transcription 6, interleukin-4 induced</td>
<td>+2.6</td>
</tr>
<tr>
<td>LTBPI</td>
<td>NM_000627</td>
<td>Latent transforming growth factor beta binding protein 1</td>
<td>+2.7</td>
</tr>
<tr>
<td>CRP</td>
<td>NM_000567</td>
<td>C-reactive protein, pentraxin-related</td>
<td>+3.0</td>
</tr>
<tr>
<td>GNAQ</td>
<td>NM_002072</td>
<td>Guanine nucleotide binding protein, G protein, q polypeptide</td>
<td>+3.1</td>
</tr>
<tr>
<td>PTH</td>
<td>NM_000315</td>
<td>Parathyroid hormone</td>
<td>+2.8</td>
</tr>
<tr>
<td>IAPP</td>
<td>NM_000415</td>
<td>Islet amyloid polypeptide</td>
<td>+4.9</td>
</tr>
<tr>
<td>BCAR1</td>
<td>NM_014567</td>
<td>Breast cancer anti-estrogen resistance 1</td>
<td>+2.9</td>
</tr>
<tr>
<td>PLCB1</td>
<td>NM_015192</td>
<td>Phospholipase C, beta 1</td>
<td>+4.6</td>
</tr>
<tr>
<td>DHDDS</td>
<td>NM_024887</td>
<td>Dehydrodolichyl diphosphate synthase</td>
<td>+2.6</td>
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<tr>
<td>VTN</td>
<td>NM_000638</td>
<td>Vitronectin</td>
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<tr>
<td>SRC</td>
<td>NM_005417</td>
<td>V-src sarcoma</td>
<td>+2.8</td>
</tr>
<tr>
<td>NP</td>
<td>NM_000270</td>
<td>Nucleoside phosphorylase</td>
<td>+3.9</td>
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aNCBIGenBank Accession number.
bObserved by array analysis as described (25): expression data were normalized to average expression levels of three housekeeping genes, namely, β-actin, GAPDH and ubiquitin.

Figure 2. BiblioSphere Pathway view network of input genes which are involved in inflammation and regulated by CoQ10. The network of five selected genes was obtained with BiblioSpherePathwayEdition software package based on co-citations with transcription factors, functional co-citations, and co-citations with other genes in the network. The abbreviations and descriptions of genes were listed in Table 1. IN, input gene; ST, gene product is part of a Genomatix signal transduction pathway; TF, transcription factor.
ACKNOWLEDGEMENTS
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REFERENCES


