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Survival transcriptome in the coenzyme Q_{10} deficiency syndrome is acquired by epigenetic modifications: a modelling study for human coenzyme Q_{10} deficiencies

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ABSTRACT

Objectives: Coenzyme Q_{10} (CoQ_{10}) deficiency syndrome is a rare condition that causes mitochondrial dysfunction and includes a variety of clinical presentations as encephalomyopathy, ataxia and renal failure. First, we sought to set up what all have in common, and then investigate why CoQ_{10} supplementation reverses the bioenergetics alterations in cultured cells but not all the cellular phenotypes.

Design Modelling study: This work models the transcriptome of human CoQ_{10} deficiency syndrome in primary fibroblast from patients and study the genetic response to CoQ_{10} treatment in these cells.

Setting: Four hospitals and medical centres from Spain, Italy and the USA, and two research laboratories from Spain and the USA.

Participants: Primary cells were collected from patients in the above centres.

Measurements: We characterised by microarray analysis the expression profile of fibroblasts from seven CoQ_{10}-deficient patients (three had primary deficiency and four had a secondary form) and aged-matched controls, before and after CoQ_{10} supplementation. Results were validated by Q-RT-PCR. The profile of DNA (CpG) methylation was evaluated for a subset of gene with displayed altered expression.

Results: CoQ_{10}-deficient fibroblasts (independently from the aetiology) showed a common transcriptomic profile that promotes cell survival by activating cell cycle and growth, cell stress responses and inhibiting cell death and immune responses. Energy production was supported mainly by glycolysis while CoQ_{10} supplementation restored oxidative phosphorylation. Expression of genes involved in cell death pathways was partially restored by treatment, while genes involved in differentiation, cell cycle and growth were not affected. Stably demethylated genes were unaffected by treatment whereas we observed restored gene expression in either non-methylated genes or those with an unchanged methylation pattern.

ARTICLE SUMMARY

Article focus

- To analyse the common gene expression profile in primary cell cultures of dermal fibroblasts from patients suffering any of the clinical presentation of the human syndrome of coenzyme Q_{10} (CoQ_{10}) deficiency (primary or secondary CoQ_{10} deficiency).
- To determine why CoQ_{10} treatment, the current therapy for all forms of CoQ_{10} deficiency, restored respiration but not all the clinical phenotypes.
- To investigate the stable genetic cause responsible for the survival adaptation to mitochondrial dysfunction owing to CoQ_{10} deficiency.

Key messages

- The mitochondrial dysfunction owing to CoQ_{10} deficiency induces a stable survival adaptation of somatic cells in patients at early or postnatal development by epigenetic modifications of chromatin. Deficient cells unable to maintain this survival state during differentiation would die contributing to the pathological phenotype.
- Supplementation with CoQ_{10} restores respiration through enhanced sugar rather than lipid metabolism; partially restores stress response, immunity, cell death and apoptotic pathways; and does not affect cell cycle, cell growth, and differentiation and development pathways.
- Survival transcriptome in the CoQ_{10} deficiency syndrome is acquired by epigenetic modifications of DNA: DNA-demethylated genes corresponded to unaffected genes by CoQ_{10} treatment, whereas those with unchanged DNA-methylation pattern corresponded to genes with responsive expression to CoQ_{10} supplementation. These results would approach to explain the incomplete recovery of clinical symptoms after CoQ_{10} treatment, at least in some patients.

ARTICLE SUMMARY

Strengths and limitations of this study

- Human CoQ_{10} deficiencies are considered rare diseases with low prevalence, which limits the sample size.
- The genetic heterogeneity of this disease is owing to mutations in any of the 11 genes directly involved in the synthesis of CoQ_{10} inside mitochondria, or other mutations altering somehow the mitochondria and its metabolism, affecting their inner CoQ_{10} synthesis as a side effect, will course with CoQ_{10} deficiency.
- Among this genetic heterogeneity, all cells showed a common transcriptomic profile that justified their pathological phenotype, responded equally to CoQ_{10} treatment and presented the same DNA methylation pattern.

Conclusions: CoQ_{10} deficiency induces a specific transcriptomic profile that promotes cell survival, which is only partially rescued by CoQ_{10} supplementation.

INTRODUCTION

Coenzyme Q_{10} (CoQ_{10}) is a small electron carrier which is an essential cofactor for several mitochondrial biochemical pathways such as oxidative phosphorylation, β-oxidation and pyrimidine nucleotide biosynthesis. CoQ_{10} biosynthesis depends on a multienzyme complex[1] that involves at least 11 proteins encoded by COQ genes. Mutations in any of these genes cause primary CoQ_{10} deficiencies, which are clinically heterogeneous mitochondrial diseases.[2] Clinical presentations include encephalomyopathy with lipid storage myopathy and myoglobinuria,[3] ataxia and cerebellar atrophy,[4] severe infantile encephalomyopathy with renal failure,[5] isolated myopathy,[6] and nephrotic syndrome.[7] Secondary CoQ_{10} deficiency has also been associated with diverse mitochondrial diseases.[8–13] In all of these conditions, CoQ_{10} supplementation partially improves symptoms[14 15] and usually induces a return to normal growth and respiration in CoQ_{10}-deficient fibroblasts.[8 16 17] Adaptation of somatic cells to CoQ_{10} deficiency may affect both onset and course of the disease. We document common transcriptomic profile alterations in somatic cells of CoQ-deficient patients, their response to CoQ_{10} supplementation, and the relationship with the DNA methylation status of specific genes.

MATERIALS AND METHODS

Cells

Primary skin fibroblasts from CoQ_{10}-deficient patients and from aged-matched controls, at similar culture passage, were cultured at 37°C using Dulbecco's Modified Eagle Medium (DMEM) 1 g/l glucose, L-glutamine and pyruvate (Invitrogen, Prat de Llobregat, Barcelona) supplemented with an antibiotic/antimycotic solution (Sigma Chemical Co, St Louis, Missouri) and 20% fetal bovine serum (FBS, Linus). When required, CoQ_{10} prediluted in FBS was added to the plates at a final concentration of 30 µM (CoQ_{10}, Synthetic Minimum 98%, high-performance liquid chromatography, Sigma). We studied five patients with primary CoQ_{10} deficiency: two siblings harboured a homozygous p.Y297C mutation in the *COQ2* gene,[5] other with a pathogenic mutation (c.483G>C) in the *COQ4* gene (this paper), and another one with haploinsufficiency of *COQ4*.[18] Patients with secondary CoQ_{10} deficiency included: a mitochondrial encephalopathy, lactic acidosis and stroke-like episodes patient harbouring the m.3243A>G in the mitochondrial $tRNA^{Leu(UUR)}$ with 43% heteroplasmy level,[8] a patient with mtDNA depletion syndrome[12] and a third patient with ataxia of unknown origin.[4] Table 1 summarises the clinical phenotype and biochemical studies of these patients.

Transcriptome analysis

RNA extraction, probe synthesis and hybridisation with two independent expression arrays (GeneChip Human Genome U133 Plus 2.0 and GeneChip Human Gene 1.0 ST, Affymetrix) were used as described.[19] Gene expression was validated by the MyiQ Single Color Real Time PCR Detection System (Biorad). See supplementary methods for full description.

Data had been deposited with the NCBI-GEO database, at http://www.ncbi.nlm.nih.gov/geo/, accession number GSE33941 (this SuperSeries is composed of two subset Series, see online supplementary table S7 for an explanation).

Statistical analyses were performed comparing each signal of patient's fibroblasts RNA with the corresponding signal of control RNA by two different approaches. The main statistical analysis for both GeneChip Human Genome U133 Plus 2.0 Array and GeneChip Human Gene 1.0 ST Array was achieved as previously described,[19] which selects the most significant genes commonly and equally regulated in all samples using very stringent parameters. In a few special cases, other unselected but regulated genes were studied because of their role in specific processes and pathways. They were equally described in table 2. The second statistical analysis approach for the Gene Ontology (GO) study was performed as previously described[20] and analyses the most altered biological processes and pathways using a lower stringency analysis, which permits to select the hundred most altered GOs in different functional categories (see online supplementary table S4) and the hundred more distorted pathways (see online supplementary table S5) that had been regulated in CoQ_{10}-deficient cells. GO regulated in both independent analysis of primary and secondary CoQ_{10} deficiencies (see online supplementary table 3), and those regulated by CoQ_{10} supplementation (see online supplementary table S9) were studied using the GORILLA software (Gene Ontology enrichment analysis and visualisation tool), at http://cbl-gorilla.cs.technion.

Table 1 Clinical phenotype and biochemical studies performed in patients with coenzyme Q_{10} deficiency

Patient/cells*	Clinical phenotype	Biochemical studies (% with respect to mean reference values)	Effect of CoQ_{10} supplementation†	Reference as cited in the text	Array and epigenetic code
Human dermal skin fibroblast	Healthy volunteers	*Reference values*	*Reference values*	[12]	#2 #HDF #control
12-year-old girl	▶ Ataxia and cerebellar atrophy ▶ Secondary CoQ_{10} deficiency	▶ 17% CoQ_{10} in muscle ▶ 31% mt-RC complex I+III (muscle) ▶ 46% mt-RC complex II+III (muscle) ▶ 22% CoQ_{10} in fibroblast ▶ 24% CoQ_{10} biosynthesis rate ▶ ROS production (three fold)	▶ Improvement of neurological assessment ▶ No biochemical studies performed	[4]	#1
33-month-old boy(his sister below)	▶ Corticosteroid-resistant nephropathy ▶ Progressive encephalomyopathy ▶ COQ2 gene mutation (c.890A>G) ▶ Primary CoQ_{10} deficiency	▶ 23% CoQ_{10} in muscle ▶ 19% mt-RC complex I+III (muscle) ▶ 32% mt-RC complex II+III (muscle) ▶ 17% CoQ_{10} in fibroblast ▶ 10% CoQ_{10} biosynthesis rate ▶ 57% mt-RC complex II+III (cells)	▶ Improvement of neurological assessment but not the renal dysfunction ▶ Recovery of cell growth ▶ Improvement of 35% complex II+III (cells)	[5] [17] [12] case 3	#3
9-month-old girl(her brother above)	▶ Corticosteroid-resistant nephropathy ▶ COQ2 gene mutation (c.890A>G) ▶ Primary CoQ_{10} deficiency	▶ 29% CoQ_{10} in fibroblast ▶ 15% CoQ_{10} biosynthesis rate ▶ 60% mt-RC complex II+III (cells)	▶ Improvement of 25% complex II+III (cells) ▶ Recovery of cell growth	[17] [12] case 4	#5
Boy	▶ MELAS (A3243G mutation) ▶ Secondary CoQ_{10} deficiency	▶ 58% CoQ_{10} in fibroblast ▶ 35% mt-RC complex I (cells) ▶ 41% mt-RC complex II+III (cells) ▶ 12% mt-RC complex IV (cells) ▶ 60% mt-$\Delta\Psi$ ▶ 70% mitochondrial mass ▶ ROS production (>2-fold) ▶ Defective autophagosome elimination	▶ Recovery of mt-RC ▶ Recovery of ATP production ▶ No ROS production	[8]	#4 #MEL+Q

Continued

Table 1 Continued

Patient/cells*	Clinical phenotype	Biochemical studies (% with respect to mean reference values)	Effect of CoQ_{10} supplementation†	Reference as cited in the text	Array and epigenetic code
10-day-old boy	▶ mtDNA depletion syndrome ▶ Neonatal encephalopathy ▶ Secondary CoQ_{10} deficiency	▶ 20% CoQ_{10} in muscle ▶ 32% mt-RC complex I+III (muscle) ▶ 19% mt-RC complex II+III (muscle) ▶ 15% CoQ_{10} in fibroblast ▶ 85% mt-RC complex II+III (cells)	▶ Improvement of 41% complex II+III (cells) ▶ Recovery of cell growth	[34]	#ELO #ELO+Q
3-year-old boy	▶ Dysmorphic features ▶ Ventricular septal defect and weakness ▶ Hypotonia and hyporeactivity ▶ Moderate mental retardation ▶ COQ4 gene deletion ▶ Primary CoQ_{10} deficiency	▶ 40% CoQ_{10} in fibroblast ▶ 44% CoQ_{10} biosynthesis rate ▶ 64% mt-RC complex I+III (cells) ▶ 58% mt-RC complex II+III (cells)	▶ Improvement in muscle tone and strength ▶ He began to speak and walk	[18]	#GIO
Girl	▶ COQ4 gene mutation (c.483G>C) ▶ Rhabdomyolysis ▶ Primary CoQ_{10} deficiency	▶ 18% CoQ_{10} in fibroblast	▶ Recovery of both complex I+III activity and growth of fibroblasts	This paper	#SIL+Q#epi
Girl	▶ Ataxia ▶ Secondary CoQ_{10} deficiency	▶ 38% CoQ_{10} in fibroblast	▶ Improvement of ATP synthesis	[12] case 1	#SOF+Q#epi

*Cultured at 37°C using DMEM 1 g/l glucose, L-glutamine, pyruvate (Invitrogen) plus antibiotic/antimycotic solution (Sigma) and 20% fetal bovine serum (FBS, Linus).
†CoQ_{10} prediluted in FBS was added to the plates at a final concentration of 30 µM (coenzyme Q_{10}, Synthetic Minimum 98%, high-performance liquid chromatography, Sigma).
CoQ_{10}, Coenzyme Q_{10}; MELAS, mitochondrial encephalopathy, lactic acidosis and stroke-like episodes; mtDNA, mitochondrial DNA; mt-RC, mitochondrial respiratory chain; ROS, reactive oxygen species.

Table 2 Differentially expressed genes in coenzyme Q_{10} deficiency

Gene symbol*	Gene title	FC†	FC‡	CoQ_{10}§	Q-RT-PCR¶	CoQ_{10}**
Mitochondrial metabolism						
C7orf55	Chromosome 7 open reading frame 55	−2.1	nc	–		
BRP44	Brain protein 44	2.0	2.3	U	8.0	−2-fold
C10orf58	Chromosome 10 open reading frame 58	−19.5	−1.6	pR		
NADH mobilisation						
CYB561	Cytochrome *b*561	−1.3	nc	O		
CYB5A	Cytochrome *b*5-A	−1.4	−1.5	U		
CYB5R1	Cytochrome *b*5 reductase 1	−1.3	nc	U		
CYB5R2	Cytochrome *b*5 reductase 2	−1.4	−1.9	U		
CYB5R3	Cytochrome *b*5 reductase 3	−1.4	−1.6	R		
CYB5R4	Cytochrome *b*5 reductase 4	−1.3	−1.6	R		
Lipid metabolism						
FDFT1	Farnesyl-diphosphate farnesyltransferase 1	−2.3	−1.5	U	−4.3	+2-fold
IDI1	Isopentenyl-diphosphate δ isomerase 1	−2.1	nc	U		
CH25H	Cholesterol 25-hydroxylase	−10.8	−3.2	O	−1.3	−3-fold
RSAD2	Radical *S*-adenosyl methionine domain containing 2	−6.8	1.4	pR		
INSIG1	Insulin-induced gene 1	−2.6	1.7	O		
LDLR	Low density lipoprotein receptor	−3.0	−1.8	pR		
SQLE	Squalene epoxidase	−2.5	nc	U		
SCD	Stearoyl-coenzyme A desaturase (δ-9-desaturase)	−3.3	nc	U		
Insulin metabolism						
CPE	Carboxypeptidase E	10.0	2.5	pR		
PAPPA	Pregnancy-associated plasma protein A, pappalysin	2.5	1.7	R	4.8	−5-fold
PCSK2	Proprotein convertase subtilisin/kexin type 2	−75.5	−4.3	O		
Other metabolism						
SCIN	Scinderin	−5.4	−1.4	O		
PYGL	Phosphorylase, glycogen; liver	−2.5	−1.6	R		
SLC40A1	Solute carrier family 40 (iron-regulated transporter)	7.6	2.9	R		
QPRT	Quinolinate phosphoribosyltransferase	−3.4	nc	R		
ATP8B1	ATPase, class I, type 8B and member 1	2.4	nc	pR		
Cell cycle						
POSTN	Periostin, osteoblast specific factor	73.8	153.9	U	238.2	−20%
VEGFA	Vascular endothelial growth factor A	2.9	nc	–		
SEMA5A	Semaphorin 5A, receptor for cell growth	3.6	1.6	pR		
AEBP1	AE binding protein 1	66.1	nc	R		
CSRP2	Cysteine and glycine-rich protein 2	5.3	1.5	R		
DOK5	Docking protein 5	6.5	1.6	U		
MID1	Midline 1 (Opitz/BBB syndrome)	3.9	4.4	U		
CHURC1	Churchill domain containing 1	3.5	nc	–		
CREG1	Repressor 1 of E1A-stimulated genes	3.0	1.3	R		
RUNX1	Runt-related transcription factor 1 (aml1 oncogene)	1.9	1.6	–		
BHLHB5	Basic helix-loop-helix domain containing; class B, 5	−6.1	−1.4	–		
IFITM1	Interferon induced transmembrane protein 1 (9–27)	−3.8	−3.7	O		
EDN1	Endothelin 1	−3.0	nc	U		
MATN2	Matrilin 2	−9.2	nc	U		
MCAM	Melanoma cell adhesion molecule	−6.7	−3.0	R	−10.9	+10%
MKX	Mohawk homeobox	−4.5	−1.5	–		
PSG6	Pregnancy specific β-1-glycoprotein 6	2.6	nc	–		
DCN	Decorin	2.0	−1.6	–		
PKP4	Plakophilin 4	2.0	1.4	U		
EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1	13.2	2.2	pR		
VCAN	Versican	2.8	2.7	–	4.6	+10%
SMARCA1	Component of SWI/SNF chromatin complex, member A1	−1.3	nc	pR		
SMARCA4	Component of SWI/SNF chromatin complex, member A4	−1.9	nc	pR		
CDK6	Cyclin-dependent kinase 6, overexpressed in tumour	1.4	2.9	U		
CDKN1A	P21, inhibitor of CDK	−9.2	−2.1	U		
CDKN1C	P57, inhibitor of CDK	−2.6	−1.3	R		
CDKN3	Inhibitor of CDK, overexpressed in cancer cells	1.9	2.7	U		

Continued

Table 2 Continued

Gene symbol*	Gene title	FC†	FC‡	CoQ_{10}§	Q-RT-PCR¶	CoQ_{10}**
CD31	Cell surface antigen	−1.8	−1.5	R		
RB1	Retinoblastoma protein	−1.4	nc	R		
E2F7	E2F transcription factor 7	3.6	nc	U		
E2F8	E2F transcription factor 8	2.2	nc	U		
FST	Follistatin	2.6	1.4	O		
Development and differentiation						
BDNF	Brain-derived neurotrophic factor	−2.9	nc	pR		
GRP	Gastrin-releasing peptide	−263.6	nc	–		
NTNG1	Netrin G1	−8.3	1.8	U		
PTN	Pleiotrophin (neurite growth-promoting factor 1)	−2.7	nc	R		
FOXQ1	Forkhead box Q1	−6.5	nc	–		
HOXA11	Homeobox A11	−4.3	−2.4	U		
HOXC9	Homeobox C9	−4.8	−2.0	U		
LHX9	LIM homeobox 9	−93.0	−1.5	U		
SP110	SP110 nuclear body protein	−2.5	nc	pR		
P2RY5	Purinergic receptor P2Y; G-protein coupled, 5	−4.4	−1.3	pR		
TSPAN10	Tetraspanin 10	−10.1	nc	–		
EPSTI1	Epithelial stromal interaction 1	−5.2	−1.4	R		
TSHZ1	Teashirt zinc finger homeobox 1	−2.8	nc	R		
KRT34	Keratin 34	−5.3	−7.6	R	−5.7	−60%
TPM1	Tropomyosin 1 (α)	−1.8	1.7	–		
FOXP1	Forkhead box P1	2.3	nc	–		
LMCD1	LIM and cysteine-rich domains 1	3.8	nc	U		
Cell resistance to stress						
CYP1B1	Cytochrome P450, family 1B and polypeptide 1	4.5	1.5	–	7.0	−5-fold
MGC87042	Similar to six epithelial antigen of prostate	12.2	–	R		
TMEM49	Transmembrane protein 49/microRNA 21	1.9	nc	–		
RAD23B	RAD23 homologue B (*Saccharomyces cerevisiae*)	2.2	nc	R		
TXNIP	Thioredoxin-interacting protein	2.0	−4.9	–		
SGK1	Serum/glucocorticoid regulated kinase 1	3.4	1.5	–		
SOCS3	Suppressor of cytokine signalling 3	−3.6	nc	R		
RHOU	Ras homologue gene family. member U	−8.3	nc	O		
Apoptosis						
AIM1	Absent in melanoma 1	−4.5	−1.4	O		
APCDD1	Adenomatosis polyposis coli down-regulated 1	−6.4	−1.8	O		
MAGED1	Melanoma antigen family D, 1	−1.7	nc	U		
MAGED4/4B	Melanoma antigen family D, 4/4B	−5.0	−1.6	U		
RAC2	Small GTP-binding protein Rac2 (rho family)	−2.3	−1.3	U		
TRIM55	Tripartite motif-containing 55	−11.7	−1.6	U		
IFI6	Interferon, α-inducible protein 6	−4.9	−1.3	R		
XAF1	XIAP associated factor-1	−3.0	−1.5	R		
TNFRSF10D	Tumour necrosis factor receptor superfamily 10D	2.4	2.6	U	15.1	+20%
SFRP1	Secreted frizzled-related protein 1	8.7	2.5	U	11.8	−2-fold
Signalling						
ARL4C	ADP-ribosylation factor-like 4C	3.8	1.6	pR		
USP53	Ubiquitin specific peptidase 53	4.2	1.7	–		
GABBR2	γ-aminobutyric acid B receptor, 2	13.8	2.0	U		
CNGA3	Cyclic nucleotide gated channel α-3	−67.3	nc	–		
GNG2	G-protein, γ-2	−4.2	1.4	pR		
HERC6	Hect domain and RLD 6	−7.4	−1.4	R		
MLPH	Melanophilin	−8.5	−1.9	R		
NCK2	NCK adaptor protein 2	−1.7	nc	–		
PARP14	Poly (ADP-ribose) polymerase family, member 14	−3.1	−1.5	–		
Immunity						
CDC42SE2	CDC42 small effector 2	−2.8	nc	–		
LY6K	Lymphocyte antigen 6 complex, locus K	−4.7	1.4	–		
GALNAC4S-6ST	B cell RAG associated protein	−17.3	−2.5	O		

Continued

Table 2 Continued

Gene symbol*	Gene title	FC†	FC‡	CoQ_{10}§	Q-RT-PCR¶	CoQ_{10}**
TNFSF4	Tumour necrosis factor superfamily, member 4	−5.9	nc	–		
TRIM14	Tripartite motif-containing 14	−4.5	nc	–		
BTN3(A2/A3)	Butyrophilin 3 (A2/A3)	−2.0	−1.3	R		
IFI27	Interferon, α-inducible protein 27	−9.8	nc	O		
IFI44	Interferon-induced protein 44	−3.3	−2.3	R		
IFI44L	Interferon-induced protein 44-like	−15.0	−1.9	R		
IFIT1	Interferon-induced protein (tetratricopeptide repeats 1)	−5.3	nc	–		
IFIT3	Interferon-induced protein (tetratricopeptide repeats 3)	−3.5	−1.7	R		
GBP1	Guanylate binding protein 1, interferon-inducible	−2.7	–	–		
ISG15	ISG15 ubiquitin-like modifier	−6.4	nc	R		
MX1	Myxovirus resistance 1	−7.4	−1.8	pR		
MX2	Myxovirus resistance 2	−6.1	−3.0	pR		
OAS1	2′,5′-oligoadenylate synthetase 1, 40/46 kDa	−5.1	−4.9	R		
OAS2	2′-5′-oligoadenylate synthetase 2, 69/71 kDa	−6.2	−1.6	R		
OAS3	2′-5′-oligoadenylate synthetase 3, 100 kDa	−3.6	−1.3	R		
OASL	2′-5′-oligoadenylate synthetase-like	−3.1	−2.6	R		
PSMB9	Proteasome subunit, β-type, 9	−1.8	nc	U		

*In italic letter, biomarkers used in several types of cancer as described by Yoo and collaborators.[28] See the text for more information.
†Full change (FC) in the comparative analysis ran with Affymetrix GeneChip Human Genome U133 Plus 2.0 Array. Values represent the FC (mean) for each gene corresponding to different patient samples (SAM analysis; R=1.5; false discovery rate (FDR)=0%). In parenthesis, FC of non-significant genes by the statistical threshold used, which were selected owing to their role in specific processes and pathways (see the text for full details). In the case of different probes selected for one gene, values represent the mean of FC for each probe (see online supplementary table S1 for full details).
‡FC in the comparative analysis ran with Affymetrix Gene Chip Human Gene 1.0 ST Array. In parenthesis, FC of non-significant genes by the statistical threshold used. Genes with no change (nc).
§Effect of coenzyme Q_{10} (CoQ_{10}) supplementation on gene expression in CoQ_{10} deficiency: unaffected genes by CoQ_{10} treatment (U); genes that restored the expression either partially (pR) or completely (R); genes with opposite regulation than in CoQ_{10} deficiency (O); and specifically regulated genes only after CoQ_{10} supplementation (S). Genes non-affected by CoQ_{10} supplementation (–). See the text and online supplementary table S8 for full details.
¶FC in gene expression analysed by quantitative real time PCR (Q-RT-PCR). See supplementary material and table S11 for primer sequence.
**Effect of CoQ_{10} supplementation on mRNA levels analysed by Q-RT-PCR. Positive values, increase on gene expression; negative values, decrease on gene expression.
AE binding protein 1, adipocyte enhancer binding protein 1; aml1 oncogene, acute myeloid leukaemia 1 oncogene; EGF-containing fibulin-like extracellular matrix protein 1, elongation factor G-containing fibulin-like extracellular matrix protein 1; small GTP-binding protein Rac2 (rho family), small guanosine triphosphate-binding protein Rac2 (rho family); SP110 nuclear body protein, specificity protein-110 nuclear body protein.

ac.il/.[21] Full description of statistical analysis be found in the supplementary material.

Epigenetic analysis

DNA (CpG) methylation analysis was performed using a base-specific cleavage reaction with bisulfite combined with mass spectrometric analysis (MassCLEAVE). For the statistical analysis, the CpGs' methylation degree for each gene was analysed with the MultiExperiment Viewer software developed by Saeed.[22] See supplementary methods for full description.

RESULTS

Transcriptome analysis

We studied skin fibroblasts from four patients with primary CoQ_{10} deficiency and three patients with secondary CoQ_{10} deficiency (table 1). We analysed the transcriptomic profiles and compared them with those of cells from age-matched control individuals, and evaluated the modifications induced by supplementation with 30 μM CoQ_{10} for 1 week to allow recovery of ATP levels.[8 16 17] A very stringent analysis selected the most significant genes displaying a common and equally altered expression in all samples (summarised in table 2 and shown with full details in online supplementary table S1). Other genes unselected by this analysis, but still abnormally expressed were also included in the study because of their role in specific processes and pathways, such as NADH mobilisation, cell cycle and immunity (see online supplementary table S1) and energetic metabolism (see online supplementary table S2). GO classification of these genes showed similar profiles when comparing independently primary-deficient and secondary-deficient fibroblasts (see online supplementary table S3). A lower stringency analysis showing the most altered biological processes and pathways selected 100 most altered GO in different functional categories (see online supplementary table S4) and 100 more distorted pathways (see online supplementary table S5) in CoQ_{10}-deficient cells. See supplementary data for description of statistical analyses.

CoQ_{10} treatment modified the specific transcriptomic profile displayed by CoQ_{10}-deficient fibroblasts (see

online supplementary tables S6 and S7). We classified genes into five groups according to the consequence of CoQ_{10} treatment on gene expression (see online supplementary table S8 for a graphical view). About 54% of probes with altered expression were unaffected by CoQ_{10} supplementation. Only 36% of probes showed partial or complete normalisation of expression and 2% showed inverse regulation (figure 1). Approximately 5% of probes were specifically altered after treatment in both deficient and non-deficient cells and 3% showed small or non-specific changes (these were not considered for further analysis). After statistical analysis, we obtained 70 altered GO with a significant p value (<0.001) and an enrichment value that represents the most altered GO within each group (see online supplementary table S9).

Data have been deposited with the NCBI-GEO database, at http://www.ncbi.nlm.nih.gov/geo/, accession number GSE33941 (see online supplementary table S10 for an explanation). The functional description of each gene was updated from the GeneCard of The Human Gene Compendium (Weizmann Institute of Science), http://www.genecards.org/. See supplementary data for a full description of genes, biological process and pathways regulated in CoQ_{10} deficiency.

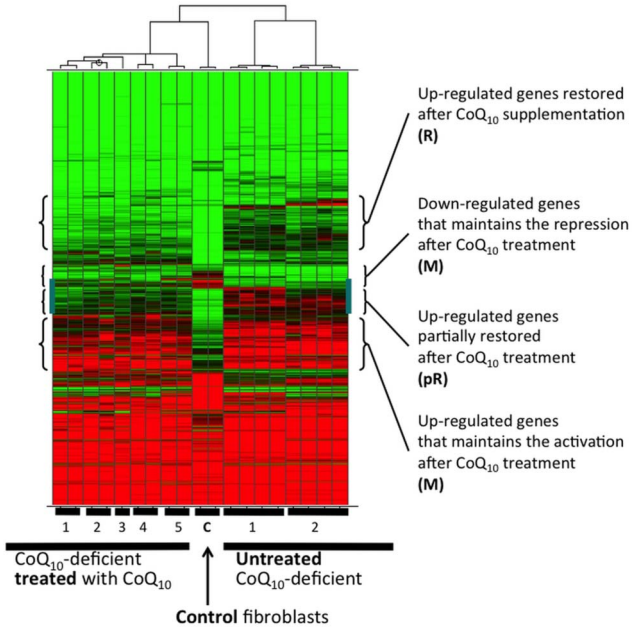

Figure 1 Cluster of genes differentially expressed in coenzyme Q_{10} (CoQ_{10})-deficiency and after CoQ_{10} supplementation. Four arrays of two representative fibroblasts from patients with Q deficiency were plotted with two arrays of control fibroblasts and nine arrays of five patient's fibroblasts with CoQ_{10} deficiency treated with 30 µM CoQ_{10}. Activated genes were coloured in red and repressed ones in green. Between parentheses—group classification of genes after CoQ_{10} supplementation (see online supplementary Table S3).

CoQ_{10}-deficient fibroblasts readapt the energetic metabolism and CoQ_{10}-treatment restores

In CoQ_{10}-deficient fibroblasts, mitochondrial functions, including respiratory chain and tricarboxylic acid (TCA) cycle, were repressed, whereas 9 of 10 steps in glycolysis and pyruvate metabolism were activated, including lactate and pyruvate dehydrogenases (see online supplementary tables S2 and S4). Accordingly, genes involved in the negative regulation of glycolysis were downregulated, whereas those involved in its activation were upregulated (see online supplementary table S2). Furthermore, genes involved in cytosolic NADH oxidation (cytochrome b_5 and several oxidoreductases) were slightly repressed (table 2). The expression of genes involved in cholesterol and fatty acid metabolism was downregulated (table 2), as well all the GO related with lipid metabolism (see online supplementary table S5).

CoQ_{10} supplementation normalised the expression (either partially or completely) of genes involved in the glycolytic pathway and activated the expression of repressed respiratory chain genes, whereas the TCA cycle remained unaffected (see online supplementary table S2). Most of the repressed enzymes of lipid metabolism and fatty acid β-oxidation remained downregulated (table 2), whereas several other pathways, such as monocarboxylic acid transport and the insulin response, were normalised (see online supplementary table S9). These results are in agreement with the recovery of aerobic metabolism observed in CoQ_{10}-deficient fibroblasts after CoQ_{10} supplementation.[8 16 17]

CoQ_{10} deficiency induces specific adaptations of cells to promote survival

The major novel finding of transcriptome profiling in CoQ_{10}-deficient fibroblasts was the altered expression of genes concerned with cell cycle and development and with resistance to stress and cell death (table 2). This suggests both a remodelling of differentiation and growth maintenance and an increase of cell survival mechanisms. Specifically, genes involved in cell cycle activation and maintenance were upregulated, and genes involved in cell cycle regulation increased or decreased their expression depending of their activating or repressing roles. This proliferative response was also enhanced by the repression of cellular attachment factors and by the activation of extracellular matrix proteins that reduce cell attachment and favour cell division. In parallel, GO clusters favouring cell cycle and cell division were activated, and those inhibiting cell growth were repressed (see online supplementary table S4). The differentiation of these cells was compromised because many required factors, transducers, antigens and structural proteins appeared downregulated, whereas repressors of differentiation during development were overexpressed (table 2). See supplementary material for a full description of genes, biological processes and related pathways.

Cell cycle activation was supported by the upregulation of CDK6 (table 2), a cyclin-dependent kinase that induces entry into the S-phase, and by a robust repression (more than ninefold) of p21/CDKN1A, an inhibitor of cyclin-dependent kinase that blocks cell cycle at the G1/S check point to stimulate cell differentiation. Moreover, subsequent pathways inactivated by p21[23] were enhanced in CoQ_{10}-deficient cells (see online supplementary table S5), as well as both transcription factors E2F7 and E2F8 (table 2), which push the progression of the cell cycle, activate cell survival and inhibit apoptosis.[24]

Cell survival in CoQ_{10}-deficient cells was improved by the induction of DNA-repairing mechanisms, and by the establishment of pathways that regulate Jun kinases and activate NAD(P)H-CoQ oxidoreductase, which are involved in stress responses (table 2 and see online supplementary table S4). Components of apoptosis and cell death pathways were systematically repressed (table 2), including tumour suppressor genes, antigens, intracellular mediators and effectors of cell death. Also, cell surface receptors and modulators that inhibit apoptosis were greatly activated.

Interestingly, CoQ_{10} treatment did not alter the newly acquired resistance to cell death in CoQ_{10}-deficient fibroblasts, kept cell growth activated, and allowed a higher degree of differentiation (tables 2 and see online supplementary table S8). However, genes controlling stress resistance pathways and cortical cytoskeleton were completely restored, as indicated by the shifts in gene expression listed in table 2. However, treated fibroblasts kept the DNA repair mechanism activated.

Signalling-related genes and pathways were differentially affected by CoQ_{10} deficiency, but most of immunity-related genes showed a general downregulation (table 2). Pathways and biological processes involved in immunity regulation were restored by CoQ_{10} supplementation (table 2 and see online supplementary table S5).

Stable DNA methylation profile is responsible for the specific gene expression profile in CoQ_{10} deficiency

CoQ_{10} supplementation modified the expression of 43% of genes that were abnormally expressed in CoQ_{10}-deficient fibroblasts (see online supplementary table S8). In the majority of these cases, expression levels were restored to those of control fibroblasts (20%), but few showed inverse regulation (2%) and others were specifically altered after CoQ_{10} treatment in both deficient and non-deficient cells (5%). The remaining 16% corresponded to partially restored genes, which slightly alter their expression level without changing the CoQ_{10}-deficient pattern. These genes along with the unaffected (54%) constitute 72% of regulated genes in CoQ_{10} deficiency, which were not significantly altered after CoQ_{10} supplementation.

To explain this differential response to respiratory dysfunction, we analysed the DNA-methylation profile of 20 among the most altered genes listed in table 2. These genes encompass the main biological processes and pathways affected by CoQ_{10} deficiency (table 3). Upregulated genes, which were unaffected by CoQ_{10} supplementation, had less-defined DNA methylation sites in their promoter regions.

Genes with partial restoration of their expression after CoQ_{10} supplementation showed precise methylation and demethylation profiles that may explain their altered expression during CoQ_{10} deficiency. The methylation degree of these genes changed after treatment, and may be responsible for the modulation of expression (table 3). The patterns of methylation of activated and repressed genes in CoQ_{10} deficiency that could be normalised by CoQ_{10} supplementation, were either unaffected or only slightly affected by the treatment, and we did not detect new methylation sites after CoQ_{10} supplementation.

However, a few genes showed significant differences in the methylation degree after the treatment, which correspond to the partially restored genes that maintain the specific expression pattern of untreated CoQ_{10} deficient cells at a lower level.

Finally, reviewing the biological processes and molecular functions of regulated genes in CoQ_{10} deficiency, the main adaptation for cell survival activated genes by DNA demethylation, which increased the expression of genes involved in cell cycle activation, apoptosis inhibition, and cell stress resistance, meanwhile the undifferentiated state could be owing to gene repression by DNA methylation, which decreased the expression of genes involved in cell differentiation. CoQ_{10} treatment did not alter the methylation degree of these genes and subsequently the expression level was maintained.

DISCUSSION

CoQ_{10}-deficient fibroblasts readapt the energetic metabolism and CoQ_{10}-treatment restores

CoQ_{10} is an essential component of the mitochondrial respiratory chain,[1] therefore dysfunctional mitochondria are a common finding in both primary CoQ_{10} deficiencies[3–7] and secondary forms.[8–13] Although each form presents a specific clinical phenotype, all these conditions display a substantial reduction of cellular CoQ_{10} content and deficit in the mitochondrial enzymatic activities of respiratory chain (table 1). Accordingly to these results, we have shown here that fibroblasts from patients with CoQ_{10} deficiency have reorganised their genetic resources to cope with this mitochondrial dysfunction. Consistent with the role of CoQ_{10} in bioenergetics, the lack of CoQ_{10} would force the cell to support it mainly by glycolysis, whereas both mitochondrial lipid metabolism and respiratory chain were repressed (see online supplementary tables S2 and S4). These findings, together with the mild repression of cytosolic enzymes that oxidise NADH (cytochrome b_5 and its oxidoreductases listed in table 2) could indicate that NADH is

Table 3 Epigenetic modifications in CoQ10 deficiency owing to DNA (CpG) methylation/demethylation

				Demethylations in CoQ_{10} deficiency			Methylations in CoQ_{10} deficiency			
Gene symbol	**FC***	**Q-effect†**	**CpGs‡**	**CpGs§**	**Degree (C/P)¶**	**CpGs' location****	**CpGs§**	**Degree (C/P)¶**	**CpGs' location****	**Q-effect††**
POSTN	73.8	U	5 (P)	2 (16 fold)	50%/3%	Close together (P)	0	–	–	–
GABBR2	13.8	U	101 (P,I)	5 (40%)	47%/37%	Close together (P)	14 (6-fold)	10%/22%	Close together (P)	–15%
VCAN	2.8	U	58 (P,E,I)	5 (2-fold)	12%/7%	Scattered groups	3 (90%)	9%/17%	Dispersed (I)	–
TNFRSF10D	2.4	U	59 (P,E,I)	27 (2-fold)	60%/25%	Scattered groups (P)	0	–	–	–
FOXP1	2.3	U	85 (I)	11 (2-fold)	57%/32%	Scattered groups	2 (3-fold)	7%/19%	Close together	–
END1	–3.0	U	25 (P)	0	–	–	0	–	–	–
PARP14	–3.1	U	29 (P)	0	–	–	3 (3-fold)	5%/19%	Dispersed	–
CPE	11.6	pR	26 (P,I)	3 (4-fold)	20%/6%	Dispersed	0	–	–	+2-fold
ARL4C	4.2	pR	63 (P,E,I)	5 (90%)	52%/28%	Scattered groups	9 (60%)	16%/28%	Scattered groups	+7%
HOXA11	–4.3	pR	17 (P)	0	–	–	8 (4-fold)	5%/20%	Close together (P)	–3-fold
AEBP1	66.1	R	80 (P,E,I)	8 (25%)	76%/61%	Scattered groups	8 (3-fold)	10%/27%	Widely dispersed	–
CYP1B1	4.7	R	24 (P)	0	–	–	0	–	–	–
CHURC1	3.5	R	20 (P,E,I)	1 (50%)	11%/7%	(I)	0	–	–	–
PYGL	–2.5	R	84 (P,E,I)	8 (2-fold)	12%/6%	Close together (E)	1 (3-fold)	4%/13%	(P)	–
XAF1	–3.0	R	25 (P,E,I)	0	–	–	0	–	–	–
EPSTI1	–5.9	R	34 (P,E)	0	–	–	7 (2-fold)	27%/37%	Scattered groups	–
MCAM	–7.7	R	74 (P,E,I)	0	–	–	0	–	–	–
MLPH	–8.5	R	8 (P)	0	–	–	0	–	–	–
PCSK2	–94.3	O	32 (P)	0	–	–	0	–	–	–
GRP	–263.6	O	73 (P,E,I)	20 (two fold)	53%/35%	Scattered groups	6 (50%)	28%/35%	Close together (P)	–

*Full change (FC) in coenzyme Q_{10} (CoQ_{10}) deficiency (patient samples (SAM) analysis; R=1.5; false discovery rate (FDR)=0%) ran with Affymetrix GeneChip Human Genome U133 Plus 2.0 Array. Full details are shown in table 2.

†Effect of CoQ_{10} supplementation on gene expression in CoQ_{10} deficiency (for more information see online supplementary table S8): unaffected genes by CoQ_{10} treatment (U), genes that restored the expression either partial (pR) or completely (R) and genes with opposite regulation after CoQ_{10} supplementation than in CoQ_{10} deficiency (O).

‡Number of CpG islands analysed. In parenthesis, gene location of CpG islands: promoter (P), first exon (E) and first intron (I).

§Significant methylated CpGs for each gene in control and CoQ_{10}-deficient fibroblast. Significance determined by t test ($p<0.01$). In parenthesis, fold change in methylation degree (small changes, in %). Non-significant changes in methylation (–).

¶Methylation degree (mean of significant CpG). Values represent the % of CpG's methylation of both control (C) and patient deficient in CoQ_{10} (P).

**Location of significant CpGs. In parenthesis, gene location: promoter (P), first exon (E) and first intron (I).

††Significant changes in CpG methylation owing to CoQ_{10} supplementation in CoQ_{10} deficiency. Positive values, an increase in the methylation degree and negative values, demethylations. Significance determined by t test ($p<0.01$) between CoQ_{10}-supplemented fibroblasts and untreated CoQ_{10}-deficient fibroblasts. Non-significant changes in methylation (–).

mainly used for biosynthetic purposes rather than for energy production.

Supplementation with CoQ_{10}, the current therapy for all forms of CoQ_{10} deficiency, restored respiration through enhanced sugar utilisation, but did not stimulate lipid metabolism. The expression of genes involved in the glycolytic pathway was partially or completely normalised after CoQ_{10} treatment, whereas the repressed genes involved in the respiratory chain were activated. The TCA cycle remained unaffected (see online supplementary table S2). These results are in agreement with the recovery of aerobic metabolism observed in CoQ_{10}-deficient fibroblasts after CoQ_{10} supplementation.[8 16 17]

CoQ_{10} deficiency induces a stable survival adaptation of cells

CoQ_{10}-deficient fibroblasts adapted several physiological processes to acquire a cellular-resistance state for survival under the conditions of mitochondrial dysfunction induced by CoQ_{10} deficiency. The new genetic pattern increases cell survival by activating cell cycle and growth, maintaining an undifferentiated phenotype, upregulating stress-induced proteins and inhibiting apoptosis and cell death pathways. These results recapitulate a survival network that can be observed in nutritional stress such as when cells are grown in galactose-enriched media.[25]

The survival adaptation shown by CoQ_{10}-deficient cells included a global resistance mechanism that is observed also during the initial phase of tumorigenesis. In fact, the CoQ_{10}-deficient expression profile was very similar to that described during myeloid cell transformation[26] and breast tumours.[27] Moreover, some of the regulated genes in CoQ_{10} deficiency (listed in table 2 as italicised letter) are used as biomarkers in several types of cancer,[28] like KRT34, the cell cycle-related POSTN, MCAM, EFEMP1 and VCAN, and the apoptotic and cell resistance-related CYP1B1, XAF1 and TNFRSF10D. Although these biomarkers behaved in CoQ_{10} deficiency (increased or decreased) as described by Yoo *et al*,[28] there is no sign of tumour formation reported in the patients so far. In addition, cellular senescence, a defining feature of premalignant tumours,[29] is characterised by a gene expression pattern similar to that of CoQ_{10}-deficient fibroblasts (see online supplementary table S5).

Supplementation with CoQ_{10} enhanced both stress response and immunity pathways. Although the pathway of cell death was partially restored, cell cycle and growth, and the mechanisms to prevent differentiation and development were not. These results indicate that the mitochondrial dysfunction owing to CoQ_{10} deficiency induces a stable survival adaptation of somatic cells in patients at early or postnatal development, and we speculate that cells unable to institute, or to maintain, this survival mechanism during differentiation will die, contributing to the pathological phenotype.

A stable DNA methylation profile is responsible of specific gene expression in CoQ_{10} deficiency

The cellular adaptation to CoQ_{10} deficiency-enhanced DNA demethylation of genes that regulate cell cycle activation, apoptosis inhibition and cell stress resistance as part of an adaptation survival mechanism. Comparable results were observed in several models of epigenetic regulation by demethylation (see online supplementary table S5), whereas DNA methylation inhibits activation of genes related to tumorogenesis and apoptosis.[30]

Pathways unaffected by CoQ_{10} treatment corresponded to stably demethylated genes, whereas those that responded to CoQ_{10} supplementation were controlled by genes with unchanged methylation patterns.

We did not find changes in the methylation degree of all genes affected by CoQ_{10} deficiency, suggesting that other modalities of gene regulation are responsible, including epigenetic mechanisms such as histone modifications by methylation and acetylation, or even DNA methylation in CpG islands other than those studied here. Interestingly, it has been reported that CoQ_{10} regulates lipid metabolism in mice liver without any effect on the DNA methylation profile,[31] indicating that supplemented CoQ_{10} by itself may not alter the DNA methylation pattern that cells acquired during the survival adaptation to CoQ_{10} deficiency.

Mechanisms unaffected by therapy corresponded to stably DNA demethylated genes, which were responsible for the acquisition of the undifferentiated state for survival and resistance that cells obtain during the adaptation to CoQ_{10} deficiency, whereas the responsive to CoQ_{10} supplementation were controlled by genes with unchanged methylation patterns and correspond mainly to metabolic genes and those related with the restoration of mitochondrial function.

We propose that these epigenetic changes may be established as early as during the fetal life[32] in order to cope with CoQ_{10} deficiency; these cells then maintain this adaptive response throughout their life. We speculate that cells unable to maintain this survival mechanism during differentiation would die contributing to the pathological phenotype.

Our model has some limits: we treated cells only for 1 week and in principle we cannot rule out that prolonged exposure to CoQ_{10} could restore also some of the other unaffected pathways. Alternatively, incomplete recovery of the gene expression profiles could be explained by the fact that exogenous CoQ_{10} can rescue the bioenergetic defect, but not all other functions of CoQ_{10} in these cells, as it has been observed in other organisms.[33]

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