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ORIGINAL ARTICLE

# Elucidation of the effect of brain cortex tetrapeptide Cortagen on gene expression in mouse heart by microarray

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**Abstract**

**OBJECTIVES**: Aging is associated with significant alterations in gene expression in numerous organs and tissues. Anti-aging therapy with peptide bioregulators holds much promise for the correction of age-associated changes, making a screening for their molecular targets in tissues an important question of modern gerontology. The synthetic tetrapeptide Cortagen (Ala-Glu-Asp-Pro) was obtained by directed synthesis based on amino acid analysis of natural brain cortex peptide preparation Cortexin. In humans, Cortagen demonstrated a pronounced therapeutic effect upon the structural and functional posttraumatic recovery of peripheral nerve tissue. Importantly, other effects were also observed in cardiovascular and cerebrovascular parameters.
**DESIGN**: Based on these latter observations, we hypothesized that acute course of Cortagen treatment, large-scale transcriptome analysis, and identification of transcripts with altered expression in heart would facilitate our understanding of the mechanisms responsible for this peptide biological effects. We therefore analyzed the expression of 15,247 transcripts in the heart of female 6-months CBA mice receiving injections of Cortagen for 5 consecutive days was studied by cDNA microarrays.
**RESULTS**: Comparative analysis of cDNA microarray hybridisation with heart samples from control and experimental group revealed 234 clones (1,53% of the total number of clones) with significant changes of expression that matched 110 known genes belonging to various functional categories. Maximum up- and down-regulation was +5.42 and –2.86, respectively.
**CONCLUSION**: Intercomparison of changes in cardiac expression profile induced by synthetic peptides (Cortagen, Vilon, Epitalon) and pineal peptide hormone melatonin revealed both common and specific effects of Cortagen upon gene expression in heart.

## Introduction

Processes associated with aging and pathologies are partially determined by the activity and competence of defense systems of the organism (such as nervous and immune) and their endogenous regulation. Targeted modulation of these mechanisms could become important means of the implementation of drug biological effects. Over the course of the last 30 years, peptide bioregulators emerged as effective means of anti-aging and immunomodulating therapy [1]. Synthetic tetrapeptide Cortagen (Ala-Glu-Asp-Pro) was obtained by directed synthesis based on amino acid analysis of natural brain cortex peptide preparation Cortexin. Previously, Cortagen had demonstrated a pronounced therapeutic effect upon the structural and functional posttraumatic recovery of peripheral nerve tissue [2], while Cortexin have demonstrated its effect upon cardiovascular and cerebrovascular parameters in human patients [3]. According to the recent report, Cortagen significantly up-regulated interleukin-2 (IL-2) gene expression in mouse splenocytes [4].

Cardiac aging is associated with progressive alterations in cardiac physiology that adversely affect the bioenergetics and function of the working myocardium [5]. Secondly, cardiovascular diseases are the major causes of morbidity and mortality in developed countries, and reach epidemic proportions in the very old (≥80 years of age). In fact, more than 10% of very old individuals will be afflicted with some form of cardiovascular disease [6, 7]. Particularly, cardiovascular aging and diseases are associated with altered patterns of gene expression, involving quantitative and qualitative changes in the abundance of specific transcripts [8]. Considering a foremost role of brain in regulation of the cardiac physiology, we hypothesized that the acute course of Cortagen treatment followed by the large-scale screening of transcripts with expression altered in heart would lead to the identification of responsive genes, therefore facilitating our understanding of the mechanisms peptide bioregulators employ to implement their biological effects. For comparative purposes the results of gerontoprotective dipeptide Vilon (Lys-Glu), tetrapeptide Epithalon (Ala-Glu-Asp-Gly) [9] and pineal peptide hormone melatonin [10] effect upon cardiac gene expression has also been used.

## Materials and Methods

Female CBA were purchased from the "Rappolovo" Animal Farm of the Russian Academy of Medical Sciences (St. Petersburg). The animals received sterilized standard laboratory chow and tap water *ad libitum*. At the age of 6 months, animals were randomly separated into 2 groups, 10 mice each. Mice of experimental group were injected s.c. with Cortagen at a single dose of 0.1 $\mu$g/animal/per day for 5 consecutive days (peptide synthesized by E.I.Grigoriev, Saint-Petersburg Institute of Bioregulation and Gerontology, RAMS). Peptide was diluted in 0.1 ml of saline. Mice injected with 0.1 ml saline served as controls. On the 6th day of the experiment, mice were sacrificed by decapitation; hearts were taken, frozen in liquid nitrogen and stored at –80°C. Total RNA was extracted from pooled samples (2–3 hearts in each) by isothiocyanate method [11].

15K cDNA clone set of the National Institute of Aging, containing library of 15,247 cDNA clones was used in the experiment [12]. Microarray manufacturing, RNA probe synthesis, $^{33}P$ labeling, hybridization and signal acquiring were performed as described [9, 10, 13]. After background subtraction, relative expression ($Z_{Trans}$) was calculated as a ratio of the Log10 Spot Value and Log10 Spot Value Average difference to the Log10 Spot Value Standard Deviation. Results from three independent hybridizations were obtained for each sample using individual probes. Experimental hybridizations were compared to control ones using $Z_{Ratio}$ (ratio of the $Z_{Tans}$ Experiment and $Z_{Trans}$ Control difference to the Standard Deviation of this difference) that was then averaged and sorted. Coefficient of variation (CV) was calculated for each membrane independently as a percent ratio of Log10 Spot Value Standard Deviation to the Log10 Spot Value Average. Coefficient of correlation ($R^2$) was calculated for each 15K clone set independently with MS Excel 97 CORREL statistical function.

The primers for RT-PCR were designed using Oligo 4.0 (Molecular Biology Insight) or Primer3 [14] software (Table 2). RT-PCR amplifications were performed using the Perkin Elmer GeneAmp PCR System 9600 with Tth Polymerase (Promega) essentially as described by the manufacturer. DNA amplifications were for 40 cycles of 1 minute at 95°C, 30 seconds at 65°C, and 30 seconds at 72°C. The final extension was 5 minutes at 72°C. Each RT-PCR experiment included one set of primers for β-Actin as a control. The "Multi-Analyst 1.1" (Bio-Rad) software was used to analyze the relative levels of the amplified gene products.

## Results

Triplicate hybridizations of cDNA microarrays of 15,247 cDNA clones were performed with probes synthesized from heart samples of mice from control and experimental groups. Controls demonstrated generally lower coefficients of variation (CVs), than treated samples (CV=14.9–16.5% for three control hybridizations; CV=18.8–19.7% for experimental group). Average coefficients of correlation were 0.935 and 0.969 for three hybridizations in control and experimental groups, respectively. Comparative microarray analyses among heart samples of mice from controls and groups administered Cortagen for 5 days, revealed a total of 252 transcripts (1.65%) with expression altered significantly ($Z_{Ratio}$ >2 or < –2; Figure 1). Within these, 5 genes were represented by more than one clone (2 to 13 clones for a single gene). Taken together, a group of 234 genes were represented by 252 clones. Among 234 non-redundant transcripts, 3 (1.3%) matched mitochondrial and 70 (29.9%) nuclear genes of the mouse, while a portion of the clones matched sequences of human genes (35 clones, 14.9%), and genes of other organisms, namely *Rattus norvegicus* and *Macaca fascicularis* (2 clones, 0.9%); 124 clones (53.0%) did not

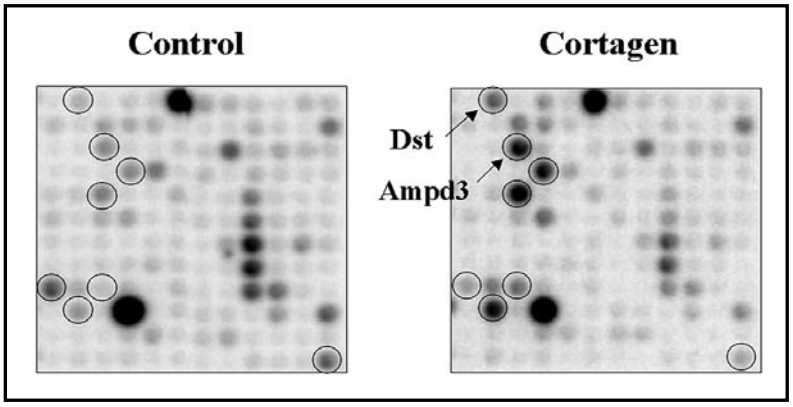


**Figure 1.** Hybridization of control and Cortagen-treated mouse heart probes with NIA 15K cDNA microarray (fragment). Circles denote clones with expression changed ($Z_{Ratio}$ values >2 or <-2). Dst, Dystonin; Ampd3, AMP deaminase H-type.

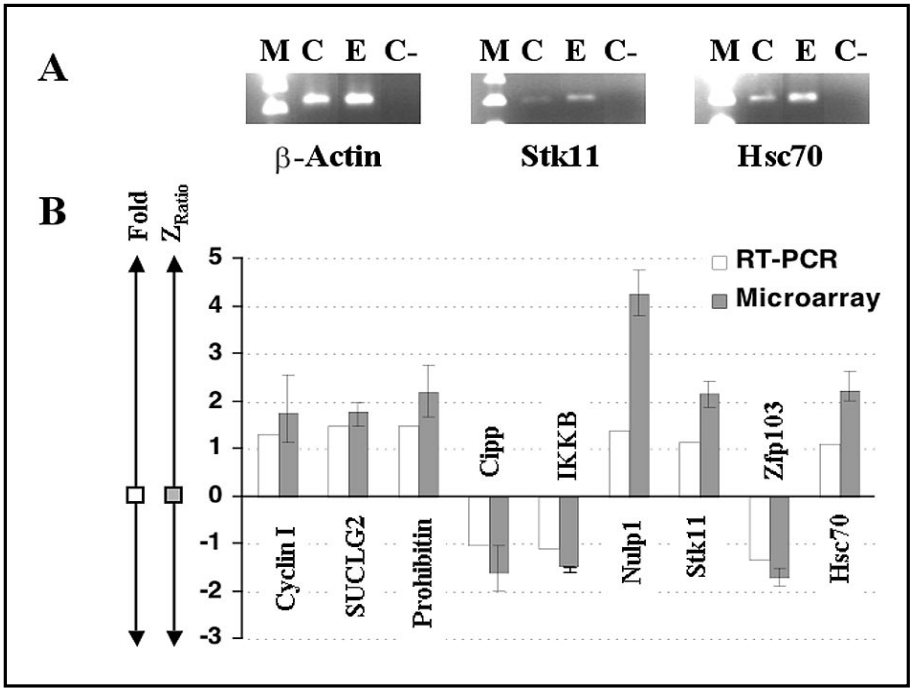


**Figure 2.** RT-PCR analysis of differentially expressed genes from cDNA microarray data. (A) Examples of ethidium bromide-stained agarose gels from which bands were quantified. M, 100 bp DNA Ladder; C, Control; E, Experiment; C-, Negative control (no template). (B) Change in the expression of randomly selected transcripts based on semiquantitative RT-PCR (Abscissa, normalized fold change) and cDNA microarray data (Abscissa, $Z_{Ratio}$). β-Actin served as a reference gene. SUCLG2, Succinyl-CoA synthetase GTP-specific β subunit; Cipp, Channel-interacting PDZ domain protein; IKKB, IkB kinase β; Stk11, Serine/threonine kinase 11; Zfp103, Zinc finger protein 103; Hsc70, Heat shock protein 70.

match any sequence in public databases. Among non-redundant transcripts, expression of 208 mRNAs was up-regulated and 26 down-regulated by Cortagen with a maximum up- and down-regulation of +5.42 and –2.86, respectively.

Nucleotide sequences of 110 transcripts with cardiac expression altered by Cortagen matched known genes, including 3 genes encoded by mitochondrial genome (16S, Cytochrome C oxidase 3 (COX3) and NADH dehydrogenase 5 (ND5)). One hundred seven nuclear genes were classified according to the functional classification of cardiovascular system-expressed genes [15]. While 31 genes were unclassified or encoded hypothetical proteins, 76 genes represented all 6 major functional categories, including genes of cell division (8), cell signaling/communication (17), cell structure/motility (6), cell/organism defense (10), gene/protein expression (19), and metabolism (16) (Table 1). Database for Annotation, Visualization and Integrated Discovery (DAVID; National Institute of Allergy and Infectious Diseases; http://apps1.niaid.nih.gov/David/upload.asp) annotated the majority (97) of genes. Functional categorization by DAVID revealed that when classified based on biological process, the majority of classified genes (43 of 56 classified) match physiological processes. Genes classified based on molecular function match mostly binding activity or enzymatic activity (39 and 26 genes of 62 classified, respectively); and genes classified based on cellular component generally matched intracellular structures (43 of 53 classified).

For validation purposes, semiquantitative RT-PCR reactions with sets of primers representing wide range of genes that demonstrated altered expression in experimental groups (9 genes with up-regulated and 3 with down-regulated expression) were performed using same RNA samples as in microarray experiment; with β-actin serving as a reference (Table 2). Selected genes represent various functional categories, including cell division, cell/organism defense, etc. While direct quantitative comparison of RT-PCR and cDNA microarray hybridization data is not possible, RT-PCR analysis demonstrated trends in gene expression levels similar to ones observed in microarray experiment for 9 genes of 12 tested (Figure 2).

Comparative analysis of changes in cardiac gene expression profiles by other short peptides (Vilon and Epithalon, [9]) and melatonin [10] revealed that the changes in the expression of many of the same transcripts were observed for every experimental group. At the same time, a relatively specific effect of Cortagen was detected for hormones and growth factors and in-

**Table 1. Effect of Cortagen on gene expression in female CBA mouse heart.**

| N | ΔE | Clone | Sp | Gene | FC |
|---|---|---|---|---|---|
| | | | | ***Cell division*** | |
| 1. | 2.94 | H3056F10 | Hs | Epidermal growth factor receptor pathway substrate 15 (Eps15) | 1a |
| 2. | 2.19 | H3145G02 | Mm | Prohibitin (Phb) | 1a |
| 3. | 2.02 | H3148A11 | Mm | Epidermal growth factor receptor pathway substrate 15, related sequence (Eps15-rs) | 1a |
| 4. | 2.25 | H3051A09 | Hs | MCM10 homolog | 1b |
| 5. | –2.86 | H3067E06 | Mm | DNA primase, p58 subunit (Prim2) | 1b |
| 6. | 3.08 | H3052B05 | Hs | Cullin 5 (LOC63330) | 1d |
| 7. | 2.09 | H3054F09 | Mm | Cyclin ania-6b geneuence | 1d |
| 8. | 2.89 | H3052C04 | Hs | APG5 (autophagy 5, S. cerevisiae)-like (APG5L) | 1e |
| | | | | ***Cell signaling/communication*** | |
| 9. | 2.58 | H3052F06 | Mm | Platelet/endothelial cell adhesion molecule (Pecam) | 2a |
| 10. | 2.01 | H3054E05 | Mm | Thrombospondin 3 (Thbs3) and mucin 1 (Muc1) | 2a |
| 11. | 2.21 | H3054H04 | Mm | K intermediate/small conductance Ca-activated channel, subfamily N, member 4 (Kcnn4) | 2b |
| 12. | –2.01 | H3098C10 | Mm | Neurocalcin-δ | 2c |
| 13. | 2.86 | H3150E09 | Mm | Bone morphogenetic protein 2 (BMP-2) | 2d |
| 14. | 2.36 | H3001A02 | Mm | Secretin (Sct) | 2d |
| 15. | 2.12 | H3001H10 | Mm | Testis-specific thymosin β-10 | 2d |
| 16. | 2.01 | H3149E05 | Mm | Angiotensinogen (Agt) | 2d |
| 17. | 2.15 | H3143C01 | Mm | Cyclic AMP phosphoprotein, 19kD (Arpp19-pending) | 2e |
| 18. | 2.13 | H3131G09 | Hs | RAS p21 protein activator (GTPase activating protein) 1 (RASA1) | 2e |
| 19. | 3.30 | H3052B11 | Mm | PCTAIRE-motif protein kinase 3 (Pctk3) | 2g |
| 20. | 2.82 | H3009E09 | Mm | TOPK | 2g |
| 21. | 2.76 | H3106F06 | Mm | UNC51.1 serine/threonine kinase (Unc51.1) | 2g |
| 22. | 2.26 | H3056E03 | Hs | FUSED serine/threonine kinase | 2g |
| 23. | 2.19 | H3052D01 | Mm | Double cortin and Ca/calmodulin-dependent protein kinase-like 1 (Dcamkl1) | 2g |
| 24. | 2.16 | H3052C12 | Mm | Serine/threonine kinase 11 (Stk11) | 2g |
| 25. | 2.22 | H3104A04 | Mm | Colony stimulating factor 3 receptor (granulocyte) (Csf3r) | 2h |
| | | | | ***Cell structure/motility*** | |
| 26. | 2.18 | H3135D11 | Mm | β -Tropomyosin 2 | 3b |
| 27. | 3.10 | H3056C01 | Mm | Formin 2 (Fmn2) | 3c |
| 28. | 2.94 | H3022F07 | Mm | Nonmuscle tropomyosin 5 | 3c |
| 29. | 2.51 | H3056C12 | Mm | Dystonin (Bpag1-n) | 3c |
| 30. | 2.15 | H3037A03 | Hs | β-Actinin | 3c |
| 31. | 3.33 | H3121F01 | Mm | Wingless-related MMTV integration site 4 (Wnt4) | 3d |
| | | | | ***Cell/organism defense*** | |
| 32. | 3.02 | H3052D11 | Mm | Adenylate kinase 2 (Ak2) | 4b1 |
| 33. | 2.65 | H3055D08 | Mm | COP1 protein (Cop1) | 4b3 |
| 34. | 2.43 | H3145H02 | Hs | Translocation protein 1 (TLOC1) | 4b3 |
| 35. | 2.30 | H3056G12 | Mm | Enigma homolog 2 (R. norvegicus) (Enh2-pending) | 4b3 |
| 36. | –2.12 | H3032G08 | Mm | Corticosteroid binding globulin (Cbg) | 4b3 |
| 37. | 2.30 | H3054D05 | Rn | Protein associating with small stress protein PASS1 (Pass1) | 4b4 |
| 38. | 2.21 | H3139E01 | Mm | Heat shock 70 protein (Hsc70) | 4b4 |
| 39. | 2.93 | H3054E04 | Mm | Major histocompatibility locus class II region | 4c |
| 40. | 2.14 | H3054E02 | Hs | Novel mRNA from chromosome 1, which has similarities to BAT2 genes | 4c |
| 41. | –2.06 | H3040B10 | Mm | Major histocompatibility locus class II region | 4c |
| | | | | ***Gene/protein expression*** | |
| 42. | –2.14 | H3112A11 | Mm | RNA polymerase I transcription termination factor 1 | 5a1 |
| 43. | 3.35 | H3056E09 | Hs | ZNF01 and HUMORFKG1B genes | 5a3 |
| 44. | 3.24 | H3056G09 | Mm | Scm-related gene containing four mbt domains (Sfmbt) | 5a3 |
| 45. | 2.67 | H3054G09 | Mm | SMAR1 | 5a3 |
| 46. | 2.24 | H3003E01 | Mm | mCASP (cux) | 5a3 |
| 47. | 2.23 | H3131F02 | Hs | MADS box transcription enhancer factor 2, polypeptide B (myocyte enhancer factor 2B) (MEF2B) | 5a3 |
| 48. | 2.07 | H3097H08 | Mm | Retinoblastoma binding protein 4 (Rbbp4) | 5a3 |
| 49. | 2.04 | H3133A12 | Mm | Zinc finger protein 61 (Zfp61) | 5a3 |
| 50. | –2.26 | H3128B04 | Mm | USF2 | 5a3 |
| 51. | 2.19 | H3047B05 | Hs | FLJ12848 fis, highly similar to mRNA for nuclear transport receptor | 5b1 |
| 52. | 2.07 | H3080D01 | Mm | Polyubiquitin C (Ubc) | 5b1 |
| 53. | –2.01 | H3068G02 | Mm | Ubiquitin conjugating enzyme 2e (Ubc2e) | 5b1 |
| 54. | 2.30 | H3124B09 | Mm | Amyloid β precursor (protease nexin II) | 5b2 |
| 55. | –2.08 | H3049A01 | Hs | SUMO-1 specific protease FKSG6 | 5b2 |
| 56. | 4.72 | H3112F10 | Mm | Ribosomal protein L3 (Rpl3) | 5b3 |
| 57. | 2.57 | H3054F04 | Mm | p70/p85 s6 kinase | 5b3 |
| 58. | 2.29 | H3126D05 | Mm | Ribosomal protein L27 (Rpl27) | 5b3 |
| 59. | 2.26 | H3112H10 | Mm | Ribosomal protein S16 | 5b3 |
| 60. | 2.22 | H3126F06 | Mm | Ribosomal protein S29 (Rps29) | 5b3 |

**Table 1. Effect of Cortagen on gene expression in female CBA mouse heart** *(Continued).*

| N | ΔE | Clone | Sp | Gene | FC |
|---|---|---|---|---|---|
| | | | | ***Metabolism*** | |
| 61. | –2.11 | H3045A08 | Mm | Alkaline phosphatase 5 (Akp5) | 6a |
| 62. | 2.04 | H3148E08 | Mm | Ornithine decarboxylase antizyme | 6b |
| 63. | 2.47 | H3122E12 | Hs | ATP synthase β subunit (ATPSB) | 6d |
| 64. | 2.15 | H3022E08 | Mm | 2Fe-2S iron-sulfur cluster binding domains containing protein | 6d |
| 65. | –2.20 | H3061H04 | Mm | ATP synthase b chain homolog | 6d |
| 66. | 2.63 | H3055C05 | Hs | Inositol 1,3,4-triphosphate 5/6 kinase (ITPK1) | 6e |
| 67. | 2.07 | H3080F03 | Mm | Inositol polyphosphate 5-phosphatase | 6e |
| 68. | 4.73 | H3147A06 | Mm | APEX nuclease | 6f |
| 69. | 2.66 | H3056E08 | Mm | AMP deaminase H-type (AMPD3) | 6f |
| 70. | 2.42 | H3018G12 | Mm | Chondroitin 4-O-sulfotransferase (C4ST) | 6g |
| 71. | 2.18 | H3090F04 | Mm | Chondroitin 4-O-sulfotransferase 2 (C4ST2) | 6g |
| 72. | 2.73 | H3147B06 | Mm | Glyceraldehyde-3-phosphate dehydrogenase (Gapd) | 6h |
| 73. | 2.30 | H3145A07 | Mm | Aldehyde reductase (Akr1A4) | 6h |
| 74. | 2.64 | H3052B06 | Mm | ATP-binding cassette, sub-family B (MDR/TAP), member 1 (Abcb1) | 6i |
| 75. | 2.61 | H3053H06 | Mm | Citrin (Slc25a13) | 6i |
| 76. | 2.12 | H3130D06 | Hs | Na,K-ATPase β subunit (ATP1B) | 6i |
| | | | | ***Unclassified Genes*** | |
| 77. | 4.60 | H3047A02 | Mm | A-Raf proto-oncogene serine/threonine-protein kinase (KRAA) | 7a |
| 78. | 4.27 | H3047H01 | Mm | Nulp1 | 7a |
| 79. | 3.15 | H3052B09 | Mm | Nuclear antigen Sp100 | 7a |
| 80. | 2.96 | H3056D02 | Hs | FLJ22439 fis | 7a |
| 81. | 2.94 | H3055D05 | Hs | KIAA0970 protein | 7a |
| 82. | 2.85 | H3056E07 | Hs | FLJ13697 fis | 7a |
| 83. | 2.76 | H3056G05 | Hs | KIAA0029 protein | 7a |
| 84. | 2.75 | H3054E11 | Hs | Phosphoinositol 3-phosphate-binding protein-2 (PEPP2) | 7a |
| 85. | 2.74 | H3052C02 | Hs | KIAA0308 gene | 7a |
| 86. | 2.60 | H3052C05 | Hs | Putative sialoglycoprotease type 2 (LOC64172) | 7a |
| 87. | 2.51 | H3024E10 | Hs | KIAA0094 gene | 7a |
| 88. | 2.46 | H3053G06 | Mm | mg53d08.r1 | 7a |
| 89. | 2.42 | H3047C01 | Hs | Hypothetical protein FLJ10914 | 7a |
| 90. | 2.42 | H3052G11 | Mm | Ganglioside-induced differentiation-associated-protein 1 (Gdap1) | 7a |
| 91. | 2.40 | H3002D05 | Hs | FLJ12166 fis | 7a |
| 92. | 2.39 | H3131G11 | Hs | DKFZp434E033 | 7a |
| 93. | 2.36 | H3151F03 | Mm | X (inactive)-specific transcript (Xist) | 7a |
| 94. | 2.31 | H3021G11 | Mm | Calreticulin (Calr) | 7a |
| 95. | 2.29 | H3022D10 | Hs | Brain acid-soluble protein 1 (BASP1) | 7a |
| 96. | 2.28 | H3056E11 | Hs | Brefeldin A-inhibited guanine nucleotide-exchange protein 2 (BIG2) | 7a |
| 97. | 2.25 | H3126B01 | Mm | ERO1-like (S. cerevisiae) (Ero1l-pending) | 7a |
| 98. | 2.18 | H3080D05 | Hs | FLJ23435 fis | 7a |
| 99. | 2.14 | H3054C10 | Hs | KIAA0699 protein | 7a |
| 100. | 2.07 | H3097C11 | Hs | FLJ20940 fis | 7a |
| 101. | 2.06 | H3095C05 | Mm | Split hand/foot deleted gene 1 (Shfdg1) | 7a |
| 102. | 2.05 | H3056H01 | Hs | MSTP028 | 7a |
| 103. | 2.04 | H3039H10 | Mm | Epithelial protein lost in neoplasm-a (Eplin) | 7a |
| 104. | 2.00 | H3047F05 | Hs | Myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to, 3 (MLLT3) | 7a |
| 105. | –2.01 | H3061F04 | Hs | HYA22 protein | 7a |
| 106. | –2.02 | H3063C10 | Mf | Brain cDNA, clone QnpA-21065 | 7a |
| 107. | –2.03 | H3060A02 | Mm | Cysteine-rich repeat-containing protein CRIM1 (Crim1) | 7a |
| | | | | ***Mitochondrial Genes*** | |
| 108. | 5.42 | H3139C10 | Mm | Mitocondrion genome, 16S | Mt |
| 109. | 2.02 | H3124G01 | Mm | Mitocondrion genome, COX3 | Mt |
| 110. | 2.00 | H3024A09 | Mm | Mitocondrion genome, ND5 | Mt |

Only named genes are present in a table.

ΔE, Altered expression ($Z_{Ratio}$); FC, Functional category; Sp, Species: Hs – *Homo sapiens*; Mm – *Mus musculus*; Rn – *Rattus norvegicus*; Mf – *Macaca fascicularis*; Mt – *Mitochondrial gene.*

**Table 2**. Primers used in a current study and correlation of RT-PCR and cDNA microarray experimental data.

| N | Gene | Primers | PCR Product | RT-PCR /Array |
|---|---|---|---|---|
| 1. | Cyclin 1 | FW: GCAAACTCTCAGCTAGGAACCT<br>RV: TACGTGCCATTTTATTGAGCTT | 354 bp | + |
| 2. | SUCLG2 | FW: CTTATATTGGGCATCCCGTGTT<br>RV: TTCTGTCCAATGGCATCTCTCA | 288 bp | + |
| 3. | Hsp84-1 | FW: TGCTCTCCTCTGTTTTCTCACT<br>RV: TTAAGGCACAGGACAGGATAGA | 307 bp | - |
| 4. | Prohibitin | FW: ATGAGCGGGTCCTGCCTTCTAT<br>RV: ATGATGGCTGCCTTCTTCTGCT | 292 bp | + |
| 5. | Cipp | FW: CCCAGCTGGAAATCATGTCT<br>RV: AGGCAACTGTTCATCCCAAC | 300 bp | + |
| 6. | IKKB | FW: GACGGAGGATGAGAGTCTGC<br>RV: TTCCTCAGCTGGAAGAAGGA | 303 bp | + |
| 7. | Nulp1 | FW: CTGCTGGAGTCCAAGAAAGG<br>RV: CGATAATCCAGCCTGCAAGT | 296 bp | + |
| 8. | Stk11 | FW: AGATTGCCAATGGACTGGAC<br>RV: GATAGGTACGAGCGCCTCAG | 299 bp | + |
| 9. | Zfp103 | FW: AAGTGGATAACTGCCCATGC<br>RV: TCACCTGTGTGGTTTCCGTA | 305 bp | + |
| 10. | Akr1A4 | FW: GCTTATAGCCCCTTGGGTTC<br>RV: TTCCCATCCACCGTAATCAT | 299 bp | - |
| 11. | Hsc70 | FW: GGAGGTGGCACTTTTGATGT<br>RV: GAGCACGGGTAATGGAGGTA | 301 bp | + |
| 12. | IGF-BP5 | FW: AAGAAAGCAAAGCGTTGGAA<br>RV: GGATGAAATCACCCTGCACT | 306 bp | - |

tracellular transducers-encoding genes (subcategories 2d, 4 genes; and 2e, 2 genes, respectively). In fact, Cortagen up-regulates expression of all transcripts that fall into these two categories and appear among those with expression changed in at least one experimental group (i.e. Vilon, Epithalon or melatonin). Among those was Secretin (Sct; Cortagen, Vilon, melatonin), RAS p21 protein activator (GTPase activating protein) 1 (RASA1; Cortagen, melatonin), Bone morphogenetic protein 2 (Bmp2), Testis-specific thymosin β10, Angiotensinogen (Agt) and 19kD cAMP phosphoprotein (Arpp19-pending; all – Cortagen only). Effect of both Cortagen and melatonin on Secretin gene expression previously detected in heart [16] is consistent with the fact that pituitary adenylate cyclase-activating polypeptide (PACAP) directly involved in melatonin metabolism belongs to the vasoactive intestinal peptide/secretin/glucagon family [17]. This supports the link between cardiac function and its physiological regulation by brain.

## Discussion

Genes with expression altered by Cortagen are functionally related to a wide range of cellular systems, representing all major physiological categories of gene expressed in heart [15]. A comparative analysis of distribution of these within functional categories and subcategories with that of genes expressed in normal heart, revealed few differences. While major functional categories of the genes (including most widely presented gene/protein expression (19 genes) and cell signaling/communication (17 genes)) were equally widely represented in untreated heart, the detailed analysis of functional subcategories revealed the majority of gene expression changes in the subgroups of the genes related to carrier proteins and membrane transport (4b3, 4 genes, 1.71% vs. 0.9% in the normal heart), and DNA synthesis and replication (1b, 5 genes, 0.85% vs. 0.52%). This can lead to the conclusion that genes related to these two subcategories could be among the most important mediators of the Cortagen biological effects.

To maintain cardiac contractile function a constant supply of ATP by mitochondria is required. Age-associated mitochondrial decay therefore affects cardiac physiology appreciably. Although the mechanisms leading to these alterations are not fully understood, they could include direct disruption of an energy supply, interferences in energy-dependent stages of $Ca^{2+}$ cardiac metabolism, and higher oxidative damage [18]. In our experiment, cardiac expression of mitochondrial genes 16S, COX3 and ND5 was up-regulated by Cortagen. Additionally, changes in expression of a number of genes involved in ionic exchange were observed for Kcnn4, Dcamkl1 and Calreticulin (up-regulated) and Neurocalcin-δ (down-regulated). Observed changes in an expression of mitochondrial genes and ionic exchange-related genes could indeed acutely affect cardiac contractility.

Though the majority of transcripts with cardiac expression altered by Cortagen could not be united to a single group based on their function, the list of these (Table 1) include a number of transcripts representing various gene functional categories that support a wide

range of complex biological effects carried out by short peptides [1]. Among most interesting targets of Cortagen in heart are genes of Pass1, Hsc70, Bmp2, Wnt4, Eps15 and Eps15-rs. Gene Pass1 expression of which was up-regulated by Cortagen is associated with mammalian small stress protein hsp27 involved in development of tolerance to stress [19]. Similarly, Cortagen up-regulated expression of Hsc70 heat shock protein, that plays an important role in myocardial protection [20, 21].

Up-regulation of Bmp2 and Wnt4 gene expression is an indicative finding, since they both belong to gene families involved in the control on programmed cell death and heart tissue formation [22, 23]. Interestingly, Cortagen also up-regulated cardiac expression of both Epidermal growth factor receptor pathway substrate 15 (Eps15) and Eps15-related sequence (Eps15-rs) involved in the regulation of mitogenic signals, normal and neoplastic proliferation [24, 25]. Although neoplastic transformation of cardiac tissue is rare, Eps15 and Esp15-rs in adults could play a role related to that of Bmp2 and Wnt4, participating in complex signaling cascades assuring cardiac cell survival.

In conclusion, we performed the first large-scale study of short peptide Cortagen effect upon cardiac gene expression. Although expression was altered in a limited group of transcripts only (1.65% of 15,247 clones tested), they matched a number of genes essential for cardiac survival. Most importantly, Cortagen affected cardiac expression of genes related to carrier proteins and membrane transport, DNA synthesis and replication, hormones/growth factors and intracellular transducers. These findings support and facilitate our understanding of the mechanisms of reported effects that Cortexin (natural predecessor of Cortagen) have demonstrated upon hemodynamic parameters in human patients [3].

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