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Tropoelastin regulates chemokine expression in fibroblasts in Costello syndrome

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ABSTRACT

Costello syndrome is a multiple congenital anomaly associated with growth and mental retardation, cardiac and skeletal anomalies, and a predisposition to develop neoplasia. Comprehensive expression analysis revealed remarkable up-regulation of several cytokines and chemokines including Gro family proteins, interleukin-1β (IL-1β), IL-8 and MCP-1 but down-regulation of extracellular matrix components including collagens and proteoglycans of skin fibroblasts derived from a Japanese Costello syndrome patient characterized by significantly reduced tropoelastin mRNA, impaired elastogenesis and enhanced cell proliferation. In contrast, decreases in these chemokines and IL-1β expression were observed in Costello fibroblastic cell lines stably expressing the bovine tropoelastin (btEln) gene and in restored elastic fibers. These results strongly suggest that the human TE gene (*ELN*) transfer could be applicable for the gene therapy of a group of Costello syndrome patients with reduced *ELN* gene expression.

Costello syndrome (MIM 218040) is a multiple congenital anomaly characterized by developmental delay, feeding problems, a coarse face, loose skin like cutis laxa, and cardiomyopathy. The patients can develop nasal papillomata, and have a predisposition for benign and malignant tumors, including rhabdomyosarcomas, neuroblastomas, and bladder carcinomas [1,2]. In recent years germline mutations in the HRAS gene have been implicated in approximately 85% of Costello syndrome cases [3]. The clinical overlapping among the Costello [3], Noonan [4], and cardiofaciocutaneous syndromes [5] is well understood, and these three syndromes are regarded as a class of disorders caused by deregulated RAS-MAPK signaling. Activation of the HRAS gene may contribute to the enhanced cell proliferation in Costello syndrome patients. However, the molecular basis of the commonly disturbed elastic fiber formation remains to be explained.

The elastic fibers in connective tissues and blood vessel walls are made of polymeric tropoelastin (ELN) surrounded by a scaffold of microfibrils consisting of glycoproteins (e.g., fibrillins and microfibril-associated glycoproteins: MAGPs) [6]. Impaired elastogenesis is accompanied by connective and cardiovascular manifestations in several human diseases, including Williams syndrome [7], supravalvular stenosis (SVAS) [7], and cutis laxa [8], which are caused by a defect of the ELN gene itself, and Costello syndrome [9]. Hinek et al. demonstrated that the defect of ELN-binding protein (EBP), an alternatively spliced variant of the lysosomal β-galactosidase gene (*GLB1*) product, causes impaired elastogenesis by interacting with the lectin-like domain of EBP and by inducing its shedding from the cell surface of fibroblasts in Costello syndrome [10,11]. Other studies involving Costello syndrome fibroblasts revealed impaired secretion of ELN, the monomeric precursor of insoluble elastin [12], and reduced ELN gene expression [13].

In this study, we performed comprehensive gene expression profiling with a DNA microarray for skin fibroblasts derived from the present Japanese Costello syndrome patient characterized by remarkable reduction of ELN gene expression without HRAS gene mutations [13], and demonstrated for the first time up-regulation

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Table 1
PCR primer sets and conditions

Target	Forward	Reverse	Annealing temp. (°C)	Product (bp)	Cycle number
Gro-γ	GCAGGGAATTCACCTCAAGA	GGTGCTCCCCTTGTTCAGTA	52	172	25
Gro-β	GCAGGGAATTCACCTCAAGA	TCAGTTGGATTTGCCATTTTTCAGC	52	178	25
Gro-α	ATTCACCCCAAGAACATCCA	CACCAGTGAGCTTCCTCCTC	50	171	27
MCP-1	AGGAAGATCTCAGTGCAGAGG	AGTCTTCGGAGTTTGGGTTTG	52	177	25
SDF-1	AGAGATGAAAGGGCAAAGAC	CGTATGCTATAAATGCAGGG	50	132	25
IL-8	TGGGTGCAGAGGGTTGTG	CAGACTAGGGTTGCCAGATTTA	52	527	27
IL-1β	AAGCTTGGTGATGTCTGG	TGAGAGGTGCTGATGTACCA	49	330	27
COL1A1	GGCGGCCAGGGCTCCGAC	AATTCCTGGTCTGGGGCACC	61	347	27
b TE	GGAATTGGAGGCATTCCCACATTTGGG	GAGCCACGCCGACTCCAGG	60	259	30
β-Actin[a]	GACAACGGCTCCGGCATGTG	CCTTCTGCATCCTGTCGGCA	60	916	25
β-Actin (for real-time PCR)[b]	CCCAAGGCCAACCGCGAGAAGAT	GTCCCGGCCAGCCAGGTCCAG	60	219	–

[a] Primer sets for RT-PCR.
[b] Primer sets for real-time PCR.

of proliferative chemokines and cytokines as well as down-regulation of collagens and proteoglycans in the fibroblasts.

Materials and methods

Patient. The patient with Costello syndrome was a 12.8-year-old girl previously diagnosed as having idiopathic hypertrophic cardiomyopathy [13].

Fibroblasts and cell culture. Primary human skin fibroblasts derived from patients and control subjects; F642, Costello syndrome [13]; F622, galactosialidosis (lysosomal protective protein/cathepsin A deficiency) (unpublished case); F643, type I sialidosis (lysosomal neuraminidase 1 deficiency) (unpublished case); F57, neuroblastoma (unpublished case); and F592 and F258, normal subjects were cultured in Ham's F-10 medium supplemented with fetal bovine serum (FBS, 10%) (Sigma, St. Louis, MO) and antibiotics at 37 °C under 5% CO_2. The research was carried out in accordance with the Declaration of Helsinki of the World Medical Association, and was approved by the ethical committee of the institution in which the work was performed.

Construction of an expression vector containing the btEln cDNA and lipofection to dermal Costello syndrome fibroblasts. btEln cDNA was excised from plasmid vector pCLneo-CMVbtEln [15], and then inserted into vector pCX-hygro [16] to prepare the expression vector pCX-hygro *btEln*. Lipofection of the pCX-hygro *btEln* to Costello fibroblasts (F642) was performed with Unifector (B-Bridge International Inc., Mountain View, CA). Drug-resistant cell strains were selected in the presence of 25 μg/ml hygromycin, and designated as strains 7-1, 27-1, 27-4, and 27-5, respectively.

DNA microarray analysis. Isolation of poly(A) mRNA from each type of fibroblast (1×10^7 cells) comparison of gene expression

Table 2
Up-regulated gene in Costello fibroblasts (F642)

Genes	Accession No.	Expression ratio
Gro-γ	M36821	27.79[a]
Gro-β	M36820	15.92[a]
Prostaglandin-endoperoxide synthase 2	MN_000963	8.92
MCP-1/SCYA2	NM_002982	8.42
SDF-1/PBSF	U16752	5.24[a]
Neprilysin	NM_000902	5.01[a]
Thrombomodulin	NM_000361	4.66[a]
Glutamine fructose-6-phosphate transaminase 2	NM_005110	4.59[a]
CD 54/ICAM1	NM_000201	4.51[a]
Plasminogen activator inhibitor, type II	NM_002575	4.25
TNFα-induced protein 6	NM_007115	3.99[a]
NPC1	NM_000271	2.88[a]
Phospholipase A2, group IVB	NM_005090	2.85[a]
EDG2	NM_001401	2.83[a]
ST3Gal III	NM_006279	2.66[a]
Prostaglandin E synthase	NM_004878	2.64
CD44	NM_000610	2.42
CD44	M59040	2.28

[a] Detected fluorescence intensity was as low as detectable limit.

Table 3
Down-regulated genes in Costello fibroblasts (F642)

Genes	Accession No	Expression ratio
Dermatopontin	NM_001937	0.03[a]
Aggrecan 1	NM_001135	0.04[a]
COL3A1	NM_000090	0.06
HS3ST3B1	NM_006041	0.06
COL1A2	NM_000089	0.08
Prostacyclin-stimulating factor	S75725	0.08
COL1A1	NM_000088	0.10
Secreted frizzled related protein 1	NM_003012	0.11[a]
COL5A2	NM_000393	0.12
Annexin II ligand	NM_002966	0.13
Testican	NM_004598	0.13[a]
Decorin	NM_001920	0.15
Milk fat globule-EGF factor 8 protein	XM_007699	0.16
Caveolin 2	NM_001233	0.17[a]
CD90/Thy-1	AF261093	0.18
Annexin A1	NM_000700	0.18
FUT8	NM_004480	0.20[a]
Integrin, beta 1	XM_005799	0.21
FGF7	NM_002009	0.22
Annexin A2	NM_004039	0.22
ADAMTS1	NM_006988	0.22[a]
ADP-ribosylation factor 4	NM_001660	0.23
COL8A1	NM_001850	0.23
Versican	NM_004385	0.23
Phosphatidic acid phosphatase type 2A	NM_003711	0.26
Caveolin 1	MN_001753	0.27
Clathrin, heavy polypeptide	NM_004859	0.29
Lumican	NM_002345	0.29
Lamican, gamma 1	NM_002293	0.30
WNT 5A	MN_003392	0.31[a]
LTBP2	NM_000428	0.32
Annexin A5	NM_001154	0.32
Thrombospondin 2	NM_003247	0.36
Cytochrome *b*-5 (CYB5), nuclear gene encoding mitochondrial protein	NM_001914	0.36
Epithelial protein lost in neoplasm beta	NM_016357	0.36
Dystroglycan 1	NM_004393	0.37
Ribophorin II	NM_002951	0.37
Calreticulin	NM_004343	0.38
Facilitated flucose transporter	NM_006516	0.38[a]
Thrombospondin 1	NM_003246	0.38
CaM1	M19311	0.40
ADAMTS2	NM_014244	0.40
Annexin A6	MN_001155	0.41
NDST1	NM_001543	0.41
CD164/sialomucin	NM_006016	0.42

[a] Detected fluorescence intensity was as low as detectable limit.