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Gene Expression Profiles in the Acutely Dissected Human Aorta

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Objectives: *heritable connective tissue abnormalities and arterial hypertension may predispose to aortic dissection. This study evaluates gene expression profiles in the acutely dissected human aorta.*
Design, Materials and Methods: *Atlas™ Human Broad Arrays I, II, and III (Clontech) were used to compare gene expression in acutely dissected (6 patients) and normal ascending aortas (6 multiorgan donors). The tissues were also compared macroscopically.*
Results: *of 3537 genes analysed, 1250 (35%) were expressed in aortic tissue. For statistical analysis we focused on 627 genes, which had an intensity > 0.95 of the mean patients or controls. Dissected and adjacent macroscopically intact aorta displayed similar gene expression patterns. On the contrary, 66 genes were expressed significantly different in dissected aorta, compared with undiseased control aorta of multiorgan donors. Genes, predominantly upregulated in dissection, are involved in inflammation, in extracellular matrix proteolysis, in proliferation, translation and transcription. Predominantly downregulated genes code for extracellular matrix proteins, adhesion proteins and cytoskeleton proteins.*
Conclusion: *our results demonstrate for the first time the complexity of the dissecting process on a molecular level. The ultimate dissection seems to be the dramatic endpoint of a long-lasting process of degradation and insufficient remodelling of the aortic wall. Altered patterns of gene expression suggest a pre-existing structural failure of the aortic wall, resulting in dissection.*

Key Words: Aortic dissection; Gene expression; cDNA array.

Introduction

Acute dissection is the most common catastrophic event affecting the aorta[1] and occurs in about 30 new cases/1 million inhabitants/year.[2,3] Recent investigations favour the theory of a vasa vasorum bleeding forming an aortic wall haematoma, as a first step, and causing an intimal tear by bursting, as second step of aortic dissection.[4]

Heritable connective tissue diseases, such as Marfan's or Ehlers–Danlos syndrome, are well known to predispose to aortic dissection.[5–7] However, in large series only 14–22% of the aortic dissections are associated with one of these heritable disorders.[8,9] Besides hypertension, the majority of acute dissections develop without any recognisable reason. Although familial aggregation of aortic dissection has been described in several studies,[10,11] little is known about the underlying genetics of the disease.

With the development of cDNA array technology simultaneous monitoring of the expression of numerous different genes in one tissue specimen has become possible.[12,13] It is a semiquantitative analysis, which provides the opportunity to study differences in gene expression between two samples. Comparison of altered gene expression patterns in diseased and undiseased tissue may help to understand the pathogenesis of a disorder. By use of membrane-based cDNA arrays, we have examined gene expression changes in dissected compared to normal aortic tissues.

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† B. T. Müller and O. Modlich evenly contributed to this study and share the first-authorship. DNA hybridisation and array data analysis were mainly done by Dr. O. Modlich. Dr. B. T. Müller covered the clinical part of the study.

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Materials and Methods

Patients

Ascending aorta specimens were obtained from six patients operated on for type A acute aortic dissection (3 men of 55, 50, and 41 years, and 3 women of 75, 57, and 66 years; mean age 57.3 years). None had Marfan's or Ehlers–Danlos Syndrome, but one female patient had a son who also suffered from an aortic dissection. Control samples were obtained from six multiorgan donors (4 men, 30, 34, 48, and 65 years, and 2 women, 14 and 45 years; mean age 39.3 years).

The study was approved by the local Ethical Committee. The patients/relatives gave their informed consents.

In addition dissected and non-dissected ascending aorta regions from the same patient, were compared in 4 patients. Tissue specimens were snap-frozen in liquid nitrogen and were stored at −70°C before total RNA extraction. Additional tissue samples were fixed in formaldehyde and embedded in paraffin for microscopic evaluation.

Total RNA isolation and cDNA probe synthesis

Total RNA from tissue specimens was extracted by the guanidine–isothiocyanate method followed by phenol/chloroform extraction using a modified protocol supplied by Atlas™ Pure Total RNA Labeling System (Clontech Laboratories, Inc., Heidelberg, Germany). Reagents from the Atlas pure total RNA labelling system (Clontech Laboratories, Inc.) were used to treat (with DNase) total RNA to eliminate potential genomic DNA contamination. Total RNA extracted from tissue samples was quantified using Eppendorf UV spectrophotometer. The quality and integrity of total RNA preparations were additionally examined by electrophoresis of 0.5 μg isolated RNA on 1% formamide agarose gel (Seakem GTG, FMC Bioproducts, Rockland, MA). For first-strand cDNA synthesis, ten to fifteen μg of native total RNA was mixed with 1 μl of coding sequences primer mix (0.02 μM; Clontech). The RNA and primers mixture was incubated at 70°C for 4 min and then at 50°C for 4 min. The master mix containing 2 μl of 5× reaction buffer, 1 μl of 10× dNTP mix (5 mM each dCTP, dGTP, dTTP, and dATP), 0.5 μl of 100 mM DDT, 3 μl of [α-^{32}P] dATP (10 μCi/μl, 3000 Ci/mmol; Amersham Pharmacia Biotech, Freiberg, Germany) and 1 μl of Moloney murine leukemia virus reverse transcriptase (100 units/μl), recombinant from *Escherichia coli* (Sigma) was then added. After incubation for 35 min at 50°C, the reaction was terminated with mixture of glycogen and EDTA (Clontech), the labelled cDNA probes were purified, and probe incorporation was assessed by scintillation counting.

Hybridisation and image analysis

Three nylon membranes (Atlas™ Human I, II, and III Arrays – Clontech Laboratories, Inc.) containing altogether 3537 cDNA spots corresponding to 3528 known human genes and nine human housekeeping genes, as well as genomic DNA spots, and positive and negative controls, were probed with labeled cDNAs by overnight hybridisation at 68°C using ExpressHyb™ hybridisation solution (Clontech Laboratories, Inc.). Shortly, radiolabelled probes were denaturated and then added to 5 ml aliquots of hybridisation buffer containing 100 μg/ml of heat-denaturated sheared salmon testes DNA (Gibco BRL, Germany). The final total probe concentration was usually 1–3 × 10^6 counts/min. For all samples, expression profiles were averaged from two repeated hybridisation experiments performed with separate array filters. We used a gene-specific primer mix and membranes from the same lot-number to exclude possible variations of cDNA probe synthesis and hybridisation. After extensive washings (three times with 200 ml of 2× saline–sodium citrate (SSC)–1% SDS solution at 68°C for 30 min followed by one wash with 200 ml of 0.1× SSC–0.5% SDS for 30 min at 68°C), membranes were additionally washed with 200 ml of 2× SSC for 10 min at room temperature, and exposed to screens of PhosphorImager (BAS-1500, Fuji, Raytest, Germany).

Array data analysis

Radioactive intensity of each spot on the membrane was linearly digitalised to grey levels in a pixel size of 100 μm^2 in a PhosphorImager reader (BAS-1500, Fuji, Raytest, Germany) and recorded using commercially available image analysis software (TINA, Fuji). Data were listed according to intensity values for all measured positions on the array. The Excel software (Microsoft) was then used to compare expression profiles derived from various samples. Before the comparison of gene expression between control and dissected aorta samples can be done, it is necessary to normalise intensities in each of the arrays which should be compared. Normalisation corrects differences in labelling and in the quantity of initial RNAs from samples examined in arrays. Normalisation among array data obtained from different samples was based on the sum of background-subtracted

signal data from all measured positions on the membrane corresponding spotted cDNAs. Such a total intensity normalisation method relies on the assumption that quantity of initial mRNA is the same for all samples. Additionally, there is assumption that some genes are upregulated in the query samples relative to the control and that others are downregulated. For all genes in the array, these changes should balance out so that total quantity of RNA that was hybridised to the array from different samples will be the same. Therefore, the total intensity measured for all spotted positions in the array should be the same for all samples which must be analysed. Under this assumption, a normalisation factor can be calculated and used to re-scale the intensity for each gene in the array.[14] Absolute values corresponding to abundance of RNA in the probe were normalised according to mean value (after normalisation the mean value of signal intensities in each array was equal to 1). This data table can be obtained at http://www.onkochemie.uni-duesseldorf.de. We used normalisation accordingly to all seven housekeeping genes detected in the array, or to beta actin gene, as well. Results for over-all number of genes expressed in either normal or dissected aorta tissues, as well as data obtained for differential gene expression, have been identical for all applied normalisation algorithms.

Hierarchical clustering

Mean values for each gene from dissected and control sample groups were calculated. Only genes with the mean expression value 0.95 or more either in patient or control group were used for further analysis ($n = 627$). Low signals on the membrane which are close to background represent an unfavourable signal-to-noise ratio, and show the low reproducibility between experiments. Ratios formed with these values may be very high even when there is no significant difference in the expression level of the corresponding genes.[15] The normalised values of 627 genes were log-transformed, and data for each gene were then mean-centred and normalised. Data for each sample were mean-centred, too. The hierarchical clustering programme developed by Eisen *et al*.[16] was then applied to group samples on the basis of overall similarities in their gene expression patterns, as well as to group genes on the basis of similarity of their expression levels in all samples. Results were displayed with the Tree View program[16] (Fig. 1). Red colour was used to show genes with expression levels greater than the mean and blue for expression levels smaller than the mean.

Statistical analysis

The unpaired Student's *t*-test was used as appropriate with significance set at $p < 0.05$ to compare dissected aorta with undissected aorta of multiorgan donors, whereas the paired *t*-test was used to compare dissected and macroscopic intact undissected aortic pieces of the same patient.

Results

Microscopic evaluation of dissected aortic specimen

Five aortic wall specimens displayed a fresh dissection, whereas one specimen showed signs of organisation and dissection and was about one week old. Five samples had signs of additional atherosclerosis. The aortas of three multiorgan donors displayed only atherosclerosis.

Intensity of gene expression

Detectable hybridisation signals were identified for 35% of the genes spotted on Human Broad array (1250 ± 300 genes) in which absolute transcript values were greater than the 2-fold background level in each separate hybridisation experiment. This number of expressed genes corresponds to all spots on the membranes whose intensity signals achieved ~50% of the mean value of expression on the array. However, mRNA species, which present at low abundance in a cell, produce signals with intensities close to background, and usually display a low signal-to-noise ratio. Since hybridisation experiments may fail to detect some of such low-expressed genes from sample to sample, we focused only on definitely expressed genes for cluster analysis and for the statistical analysis of the differential gene expression between controls and patients' samples. Therefore, a set of 627 genes (about 17.7% of the 3537 genes analysed), whose mean transcript levels achieved at least 95% of the mean value of expression intensities, has been analysed. Additionally, the results of two independent array hybridisations performed for identical samples showed good reproducibility with correlation coefficients of 0.98 (data not shown).

Hierarchical clustering

Hierarchical clustering was applied to group the tissue samples and genes using the cluster programme.[16] The results for all hybridisation experiments are

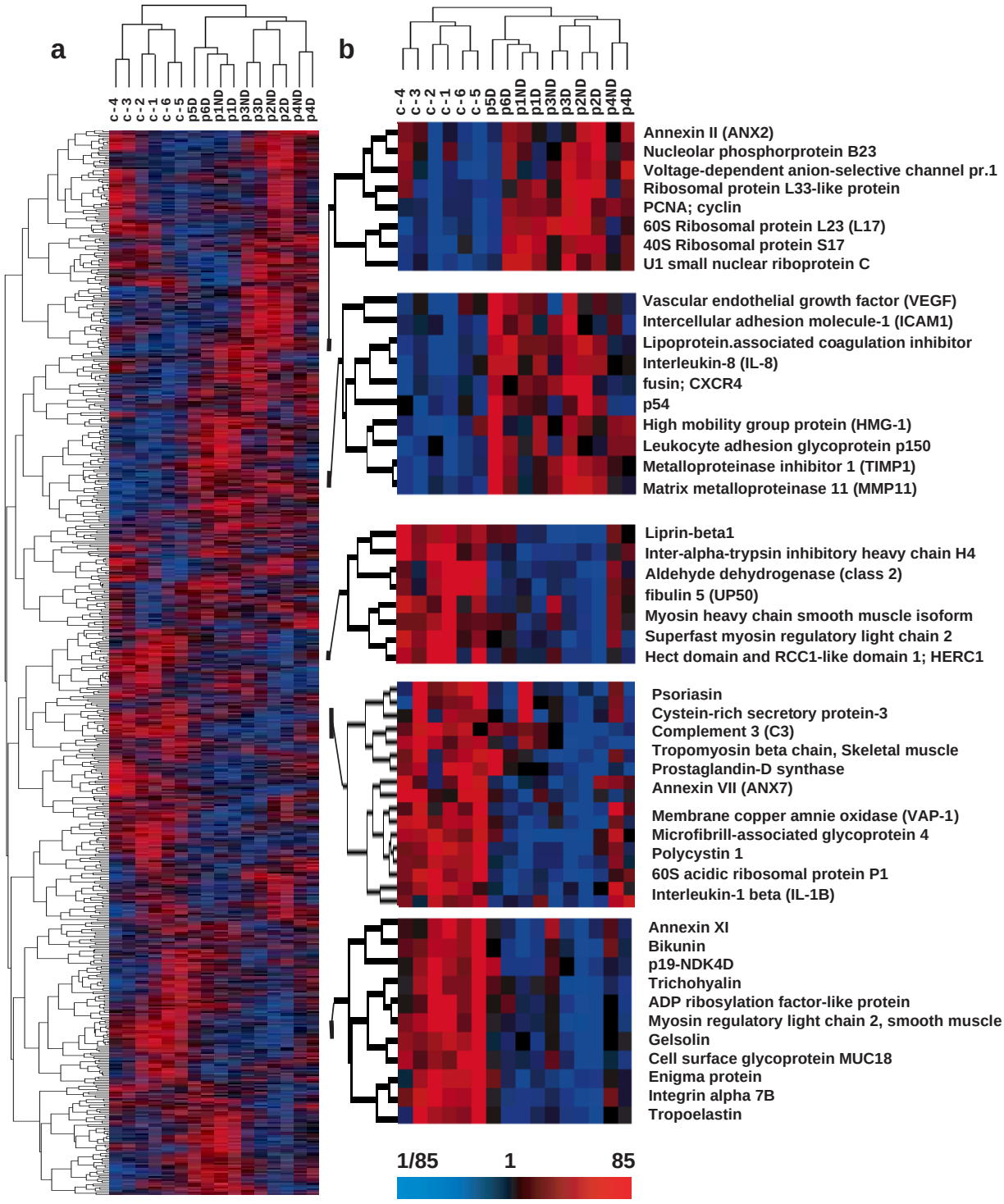

Fig. 1. Cluster diagram of 16 aortic tissue samples based on 627 expressed genes. (a) all 627 expressed genes (b) only significantly different expressed genes. c-1 to c-6 are control tissue specimen of multiorgan-donors, p1D to p6D (patient dissected) are dissected aortic tissue samples from patients. p1-4 ND (patients non-dissected) are macroscopic intact aortic parts of patients with dissection. Each row corresponds to a gene, each column to a tissue sample. Red colour shows gene with expression levels greater and blue colour with gene expression smaller than the mean.

represented as relative values for 627 selected genes on Fig. 1 (see also Material and Methods). Each column corresponds to a tissue sample and each row to a gene. Control tissue samples and samples from patients with dissection are placed on different branches of the dendrogram. Thus, hierarchical cluster algorithm using a small set of experimental expression data (627 genes), was able to distinguish between control and dissected tissue. Moreover, tissue samples taken from dissected aortic wall and from macroscopic intact wall from the same patient were clustered together. This means that independent samples taken from the same aorta were more similar to each other than to any of the other samples.

Patterns of gene expression

Dissected and macroscopic intact aortic samples of the same patients, who underwent operation for acute aortic dissection, did not show any significant differences in their gene expression pattern. On the contrary 66 of 627 genes (~10.5%) in dissected aorta compared to intact control aorta of multiorgan donors were significantly differently expressed ($p < 0.05$). Thirty-four genes (~5.4%) were expressed at higher levels in dissected aorta, and thirty-two genes (~5.1%) were expressed at lower levels in dissected aorta. Lists of differently expressed genes are summarised in Tables 1 and 2. Representative cDNA array images of

Table 1. Gene groups with predominant downregulation in dissected aorta.

Functional group (gene/protein) gene	*p*-value	*Mean ± SEM				
		Patients		Controls		Regulation
Extracellular matrix proteins, cell surface antigens and adhesion proteins						
Integrin alpha 7B	<0.001	0.44	*0.21*	1.65	*0.44*	↓
Membrane copper amine oxidase (VAP-1)	<0.001	0.57	*0.19*	1.35	*0.22*	↓
Fibulin-1, isoform C	<0.001	5.19	*1.11*	13.17	*2.65*	↓
Microfibril-associated glycoprotein 4	0.001	2.44	*0.70*	6.50	*1.97*	↓
Fibulin-5 (UP50)	0.005	0.54	*0.25*	1.15	*0.33*	↓
Polycystin precursor	0.010	1.19	*0.32*	3.09	*1.43*	↓
Selenoprotein P	0.010	6.93	*3.24*	18.98	*8.69*	↓
Cell surface glycoprotein MUC18; CD146 antigen	0.013	3.09	*1.07*	8.73	*4.48*	↓
Elastin (Tropoelastin)	0.033	0.92	*0.40*	2.80	*1.81*	↓
Fibronectin receptor alpha subunit (FNRA); Integrin alpha 5 (ITGA5); VLA5; CD49E antigen	0.030	3.30	*2.30*	0.86	*0.55*	↑
Leukocyte adhesion glycoprotein p150, Integrin alpha X (ITGAX)	0.022	2.06	*1.32*	0.58	*0.22*	↑
Cell motility and cytoskeleton proteins						↓
Myosin regulatory light chain 2; smooth muscle isof.	0.001	27.37	*12.03*	80.00	*22.88*	↓
Alpha-actinin 1 cytoskeletal isoform	0.001	0.39	*0.10*	0.80	*0.20*	↓
Superfast myosin regulatory light chain	0.002	1.20	*0.55*	3.18	*1.07*	↓
Enigma protein	0.004	2.90	*0.97*	5.70	*1.59*	↓
Kinesin-related protein	0.008	0.42	*0.14*	1.02	*0.425*	↓
Gelsolin	0.013	0.86	*0.41*	2.82	*1.53*	↓
Tropomyosin alpha chain, skeletal muscle	0.027	2.95	*1.20*	6.76	*3.37*	↓
Metabolism						
Aldehyde dehydrogenase 5; ALDH5	0.001	0.41	*0.19*	1.41	*0.53*	↓
Alcohol dehydrogase 6 + aldehyde dehydrog. 1	0.001	0.57	*0.18*	1.08	*0.21*	↓
Ornithine aminotransferase	0.002	1.04	*0.31*	1.68	*0.18*	↓
Mitochond. aldehyde dehydrog. (class 2); ALDHI	0.006	5.99	*1.99*	12.03	*3.78*	↓
Aldehyde dehydrog. 2; ALDH2	0.006	4.60	*1.01*	8.45	*2.51*	↓
cAMP-dependent protein kinase type I β regulatory subunit (PRKAR1B)	0.034	1.13	*0.45*	3.07	*1.88*	↓
Electron transfer flavoprotein α-subunit (alpha-eft)	0.025	2.43	*0.84*	1.32	*0.60*	↑
ATP synthase B chain, mitochondrial	0.004	4.21	*1.42*	1.83	*0.62*	↑
Others						
Cysteine-rich protein 1 (CRP1)	0.002	0.61	*0.25*	1.50	*0.46*	↓
Tryptophan 5-hydroxylase (TRPH)	0.004	0.40	*0.26*	1.40	*0.65*	↓
ADP ribosylation factor-like protein	0.008	0.94	*0.41*	1.91	*0.58*	↓
Gem; induced immediate early protein; ras family member (ras-like protein KIR)	0.016	1.94	*0.65*	5.08	*2.58*	↓
Inter-alpha-trypsin inhibit heavy chain H4 (PK120)	0.009	0.39	*0.22*	1.09	*0.48*	↓
CX3C chemokine	0.017	0.94	*0.42*	1.83	*0.64*	↓
Dihydropyridine-sensitive L-type, calcium channel alpha-2/delta subunits	0.019	1.47	*0.55*	6.01	*3.96*	↓
Fibrinogen B beta polypeptide	0.025	0.46	*0.19*	1.16	*0.62*	↓

↑ gene expression upregulated, ↓ gene expression downregulated in dissected aorta.
* Mean expression levels and SEM in dissected aorta and in control aorta.

Table 2. Gene groups with predominant upregulation in dissected aorta.

Functional group (gene/protein) gene	*p*-value	*Mean ± SEM				
		Patients		Controls		Regulation
Extracellular matrix proteolysis						
Matrix metalloproteinase 11 (MMP11)	0.002	10.38	*3.36*	4.37	*1.18*	↑
Metalloproteinase inhibitor 1 (TIMP1)	0.001	14.55	*5.03*	5.21	*1.44*	↑
Inflammation						
Interleukin-8 (IL-8)	<0.001	1.46	*0.49*	0.29	*1.18*	↑
Interleukin-6 (IL-6)	0.001	14.07	*5.82*	3.56	*0.95*	↑
Annexin I (ANX1)	0.001	36.72	*8.86*	15.91	*6.88*	↑
Leukocyte surface CD53 antigen	0.010	2.33	*1.04*	0.93	*0.24*	↑
Stromal cell derived factor 1 receptor; fusin	0.020	1.04	*0.51*	0.43	*0.16*	↑
Macrophage inflammatory protein 2 (MIP2-alpha)	0.034	2.25	*1.20*	0.94	*0.52*	↑
Ribosomal proteins and translation control						
U1 Small nuclear riboprotein C	0.001	1.10	*0.23*	0.62	*0.09*	↑
Ribophorin II	0.002	2.95	*0.81*	1.44	*0.36*	↑
Eukaryotic translation initiation factor 1A	0.002	1.63	*0.21*	1.09	*0.24*	↑
40S Ribosomal protein S17	0.002	2.86	*0.65*	1.61	*0.39*	↑
60S Ribosomal protein L40(CEP52)	0.003	24.21	*6.66*	12.97	*2.73*	↑
60S Ribosomal protein L23 (LI7)	0.004	27.97	*6.59*	17.07	*2.65*	↑
Elongation factor tu, mitochondrial (P43)	0.010	3.18	*1.15*	1.69	*0.18*	↑
Eukaryotic translation initiation factor 2 alpha subunit	0.022	3.13	*1.62*	1.31	*0.28*	↑
Proliferation and transcriptional control						
Ribosomal protein S6 kinase II alpha1 (S6KII-alpha 1	<0.001	0.95	*0.23*	0.39	*0.08*	↑
Ets domain protein elk-3	0.003	2.14	*0.73*	0.94	*0.18*	↑
High mobility group protein (HMG-I)	0.004	2.71	*0.48*	1.75	*0.39*	↑
E2F dimerisation partner 1; DP1	0.006	1.57	*0.37*	0.97	*0.20*	↑
Proliferating cyclic nuclear antigen (PCNA); cyclin	0.008	1.01	*0.39*	0.43	*0.17*	↑
Insulin-like growth factor-binding protein 3 (IGFBP3)	0.009	6.42	*3.68*	1.42	*0.86*	↑
DNA-directed RNA polymerase II 4.5 kD polypeptide	0.016	2.68	*1.02*	1.45	*0.16*	↑
Transforming protein rhoB	0.002	3.89	*1.10*	8.37	*2.32*	↓
Human insulin-like growth fact bind protein (IGFBP5)	0.005	1.49	*0.51*	4.62	*2.09*	↓
Others						
Adipophilin (adipose lipid metabolism)	<0.001	1.21	*0.33*	0.41	*0.15*	↑
Oncostatin M (OSM)	0.004	3.15	*0.93*	1.55	*0.50*	↑
HRIHFB2017 protein	0.005	2.41	*0.98*	0.91	*0.23*	↑
Guanine nucleotide-binding protein G(K) alpha 3 sub	0.007	3.99	*1.16*	1.93	*0.95*	↑
Antagonist decoy receptor for TRAIL/APO2L (TRID)	0.012	1.94	*0.88*	0.76	*0.34*	↑
Nuclear receptor-related 1	0.018	3.09	*1.29*	1.23	*0.95*	↑
Reticulocalbin	0.013	2.35	*0.82*	1.12	*0.55*	↑

↑ gene expression upregulated, ↓ gene expression downregulated in dissected aorta.
* Mean expression levels and SEM in dissected aorta and in control aorta.

control and dissected aortic samples are presented in Fig. 2.

Gene groups with predominant downregulation in dissected aorta

Genes with lower expression levels in dissected aorta tissue samples could be subdivided into several functional groups. Several genes that encode extracellular matrix proteins (ECM) displayed lower expression in dissected aortic wall relative to normal aortic tissue (Table 1). Whereas the elastin mRNA expression level was decreased in dissected samples, procollagen alpha 2 (IV) subunit, collagen alpha 1 (XV) chain precursor, and collagen alpha 1 (VI) chain genes were highly expressed at the same level in control and dissected tissue. Collagen 10 alpha 1 subunit (COL10A1), collagen alpha 1 (X) subunit, and collagen alpha 5 (IV) chain mRNAs were not detected on the array, as well as laminin mRNA. Levels of fibrillin 1 and 2 mRNAs were not changed. Levels of mRNA coding for cell–cell adhesion molecules, such as cadherins, catenins, and integrins were similar in dissected tissue compared to controls, whereas expression of fibulin 1 and 5 and integrin alpha 7B gene was significantly lower in the dissected aorta.

The second group of genes with decreased expression levels consists of cytoskeleton genes and myofibrillar genes. We found that expression of alpha-actinin 1 gene, two of myosin regulatory chains, as well as tropomyosin alpha chain, kinesin-related protein, gelsolin and enigma genes was downregulated in dissected aorta compared to control aorta.

Gene groups with predominant upregulation in dissected aorta

Expression levels of several genes associated with inflammation processes, e.g. the inflammatory chemokines, IL-6 and IL-8, were higher in dissected aorta. Moreover, we observed moderate differences in IL-6 and IL-8 gene expression levels in dissected compared to macroscopic intact specimens of the same aortic wall in the patient group of samples. To our surprise, only gene expression of metalloproteinases (MMP) 11 and 14 and its tissue inhibitor of metalloproteinases 1 (TIMP1) could be seen on the array, whereas signals corresponding to RNAs of MMP 2, 3, 9, 12, and 13 genes were not detected. In dissected aorta expression of MMP11 and TIMP1 genes was significantly upregulated.

Cells of damaged tissue in the dissected aorta seem to proliferate faster than in normal aorta. We found an elevated expression of many genes participating in cell growth and proliferation, as well as some transcription and translation factors in dissected tissues. Additionally, some of the ribosomal genes were upregulated in dissection, whereas production of others remained unchanged.

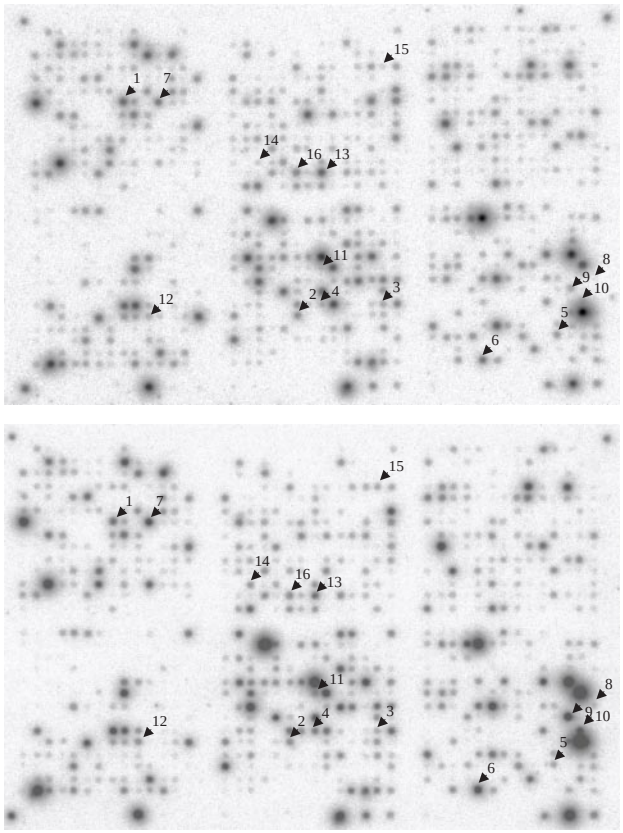

Fig. 2. Representative cDNA array images of the expression patterns in control (A) and dissected (B) aorta samples. Hybridisation of identical Human Broad I cDNA arrays was performed. The 1176 cDNA fragments of known genes are spotted on each membrane in 6 fields. Nine housekeeping genes, genomic DNA and negative controls are located in the bottom row. A complete list of gene names and their locations is available at Clontech's Web page (http://atlas.clontech.com). Spots 1 (cell surface glycoprotein MUC18; CD146 antigen); 2 (polycystin precursor; autosomal dominant polycystic kidney disease protein 1); 3 (integrin alpha 7B precursor); 4 (fibronectin receptor alpha subunit (FNRA); integrin alpha 5; CD49E antigen); 5 (inter-alpha-trypsin inhibitor heavy chain H4 precursor (ITI heavy chain H4); plasma kallikrein-sensitive glycoprotein 120); 6 (metalloproteinase inhibitor 1 precursor (TIMP1)); 7 (matrix metalloproteinase 11 (MMP11); stromelysin 3); 8 (interleukin-8 precursor (IL-8)); 9 (insulin-like growth factor-binding protein 3 precursor (IGFBP3)); 10 (interleukin-6 precursor (IL-6)); 11 (high mobility group protein (HMG-I)); 12 (ets domain protein elk-3); 13 (transforming protein rhoB); 14 (ribosomal protein S6 kinase II alpha 1 (S6KII-alpha 1)); 15 (stromal cell derived factor 1 receptor; fusin); 16 (Gem; induced immediate early protein; ras family member (ras-like protein KIR)) correspond to differentially expressed genes.

Discussion

Arrays containing cDNA fragments corresponding to 3537 human known genes have been used to detect changes in gene expression underlying Stanford A aortic dissection. This may help us to identify persons at risk, and who could profit from regular aortic screening in order to prevent emergency surgery.

Derangement or loss of some structural elements of the media is usually observed in patients with aortic dissection.[17,18] Degenerative changes within the media could affect all of its components. Whereas the content of collagen was unchanged, the elastin gene mRNA was decreased in dissected samples. Additionally, gene expression levels of microfibril-associated protein 4 (MFAP4), fibulin 1 and 5 and several secreted or transmembrane glycoproteins, as CD146 antigen, selenoprotein P were decreased in dissected tissues. They play a role in cell adhesion and control the integrity of extracellular matrix.[19,20]

There is one interesting candidate gene that seems to be involved in aortic dissection and was downregulated in dissected samples compared with normal aorta in the present study – polycystin. This gene encodes a large membrane-associated glycoprotein which is thought to contain a number of extracellular protein motifs. It functions as a matrix receptor to link the extracellular matrix to the actin cytoskeleton via focal adhesion proteins.[21,22] It is known that mutations of this gene cause autosomal dominant polycystic kidney disease. Familiar clustering of aortic dissection, supra-aortal dissection or cranial aneurysm in polycystic kidney disease have already been described.[23,24] Griffin *et al*. studied immunostaining

of smooth muscle cells using an antiserum against the polycystin protein.[25] They observed variable intensities for polycystin in tissues of intracranial aneurysms, aortic dissections, and dolicho-ectatic arteries taken from several patients with autosomal dominant polycystic kidney disease. The authors suggested that gene expression of mutant polycystin protein in arterial smooth muscle cells can be directly connected with arterial disorders often observed in this disease.

Consequently, these results suggest that some functions of extracellular matrix are changed in the wall of dissected aorta. Whereas the main structural components of the extracellular matrix remain unchanged, several transmembrane and communication proteins show decreased gene expression levels. Therefore, many important tissue functions, for example, response to injury, may be disturbed.

The second group, we found to be downregulated in dissected tissues, consists of genes encoding for cytoskeleton proteins and myofibrillar proteins. The cytoskeleton is a cytoplasmatic system of fibres supporting the cell membrane and producing movements of organelles and other elements in the cytosol. Actin is one of the most abundant intracellular proteins in the eukaryotic cell and plays an important role in cell locomotion. Gene expression of alpha actinin, an actin-binding protein, which organises actin into bundles and networks,[26] was significantly decreased in dissected aorta. Several members of the myosin gene family, the so-called motor proteins, showed decreased expression levels in dissected tissues. We conclude that, in dissected aorta, cell motility seems to be disturbed.

Dissection is clearly associated with three closely related processes, such as degradation of the aortic wall, inflammation, and cell proliferation. Expression of several genes involved in cell proliferation and transcriptional control was elevated in dissected aorta (Table 2). Metalloproteinases are known to degrade very specifically connective tissue contents. They are secreted as inactive precursors by smooth muscle cells, fibroblasts and endothelial cells and are directly controlled by their endogenous tissue inhibitors.[18,27] In one recent study, differences in gene expression in normal aorta and abdominal aortic aneurysms (AAA) were studied using the first of three cDNA arrays that were also used in our study.[28] In their study several genes were upregulated in AAAs, as MMP9 and IL-8. IL-8 is known to regulate the gene expression of MMP9.[29] In our study, expression of MMP9 was detected neither in normal nor in dissected aorta, although expression of IL-8 was significantly elevated. Although elevated levels of MMP1, MMP2, and MMP9 proteins were already demonstrated in aortic dissection using immunohistochemistry,[30] we failed to detect hybridisation signals of these MMPs on the array. Probably, quantities of MMP1, MMP2, and MMP9 mRNAs are not sufficient for their detection on arrays. Additionally IL-6, which stimulates expression of MMP11,[31] was upregulated in dissected samples, as well as MMP11 itself, also known as stromelysin 3. It is an unusual metalloproteinase, which degrades basement membrane components (collagen IV and glycoproteins as laminine and fibronectin) and ground substance as proteoglycans and gelatine.[18,32] It is inhibited by TIMP1,[18] which was also upregulated in our series. Our observation that gene expression of the basement membrane and ground substances degrading MMP11 was elevated are similar to those of Sariola *et al.*,[33] who analysed dissected aorta by immunohistochemistry. They found no defect in the expression of collagen type I and III, whereas defects in collagen IV around smooth muscle cells, as well as defects in smooth muscle basement membranes, were frequent. Thus, in the pathogenesis of aortic dissection, local changes in the basement membranes of the medial layer may be important.[33] Additionally, interruption of vasa vasorum basement membranes in the outer third of the aortic wall might play a role in vasa vasorum bleeding forming an aortic wall haematoma, as first step of dissection.

In this study tissue samples harvested during emergency aortic root replacement from patients with aortic dissection, and intact aortic samples from multiorgan donors were used. A possible limitation for the interpretation of the experimental results may be related to the different age of patients and multiorgan donors in the control group. On the average, the donors were about 40 years old, whereas the patients were about 18 years older. Therefore, we have recently started collecting tissue samples of older donors and younger dissection patients for a complementary study.

Summary

cDNA array technique was applied to analyse changes in gene expression in acute Stanford A aortic dissection. Similar gene expression patterns in adjacent macroscopic intact and dissected aortic parts of the same patient suggest that gene expression changes found in dissection compared to intact aorta of multiorgan donors develop before the dissection takes place. Dissection seems to be the dramatic endpoint of a longer lasting process of degeneration and insufficient remodelling of the aortic wall. An underlying genetic defect(s) resulting in a structural failure of

the aortic wall might be responsible for dissection. Distinguishing genes being directly responsible for dissection from those reacting on the dissecting process presents a challenge for future research.

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