Research Article

Gene Expression Changes in Venous Segment of Overflow Arteriovenous Fistula

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Aim. The objective of this study was to characterize coordinated molecular changes in the structure and composition of the walls of venous segments of arteriovenous (AV) fistulas evoked by overflow.

Methods. Venous tissue samples were collected from 6 hemodialysis patients with AV fistulas exposed to overflow and from the normal cephalic veins of 4 other hemodialysis patients. Total RNA was extracted from the venous tissue samples, and gene expression between the 2 groups was compared using Whole Human Genome DNA microarray 44K. Microarray data were analyzed by GeneSpring GX software and Ingenuity Pathway Analysis.

Results. The cDNA microarray analysis identified 397 upregulated genes and 456 downregulated genes. Gene ontology analysis with GeneSpring GX software revealed that biological developmental processes and glycosaminoglycan binding were the most upregulated. In addition, most upregulation occurred extracellularly. In the pathway analysis, the TGF beta signaling pathway, cytokines and inflammatory response pathway, hypertrophy model, and the myometrial relaxation and contraction pathway were significantly upregulated compared with the control cephalic vein. Conclusion. Combining microarray results and pathway information available via the Internet provided biological insight into the structure and composition of the venous wall of overflow AV fistulas.

1. Introduction

Arteriovenous (AV) fistulas are very useful for determining optimal blood flow for dialysis, but AV fistulas exposed to overflow are thought to increase cardiac output and cause high-output cardiac failure [1, 2].

Measurement of blood flow via an internal shunt was first developed by Krivitski et al., and the monitoring of blood flow via a shunt has since become widespread [3]. We use this technique to monitor the blood flow of AV fistulas at our hospital and correct overflow AV fistulas with surgery.

It is thought that the outflow vein of overflow AV fistulas bears a heavy load: as the vein is exposed to increased arterial flow, the wall dilates, triggering a vascular remodeling process. However, the molecular mechanisms by which the outflow vein is remodeled into a mature fistula remain unclear. By investigating venous remodeling in overflow AV fistulas, candidate genes important to the remodeling process can be discovered and their functional significance investigated. Thus, the identification of relevant genes involved in this process should provide insight into AV fistula maturation.

In this study, we performed a cDNA microarray analysis and compared segments of the venous walls of overflow AV fistulas from 6 hemodialysis patients with the normal cephalic veins of 4 other hemodialysis patients to determine whether there was any difference in their gene expression patterns.

2. Study Population

From June 2009 to September 2010, 548 patients underwent hemodialysis at the Oyokyo Kidney Research Institute in Hirosaki, Japan. During that period, 10 patients underwent surgical ligation to correct an overflow AV fistula. When the operation was performed, we retained a sample of the wall of
the overflow AV fistula (Figure 1). The AV fistula specimens were resected from the wall of the vein close to the AV fistula anastomosis. The study was approved by the Bioethics Committee of Oyokyo Kidney Research Institute, and all patients provided their informed consent to the procedure prior to it being performed.

3. Inclusion Criteria

The inclusion criteria were as follows: (1) blood access flow greater than 2.0 L/min measured by the color Doppler ultrasound (2) an AV fistula in the lower arm with a distal radiocephalic anastomosis. In total, 6 patients had overflow AV fistulas that met these criteria. The backgrounds of these patients are detailed in Table 1. We also obtained tissue samples from the lower arm distal cephalic veins of 4 new hemodialysis patients and used these as a control.

4. Methods

As noted above, venous tissues were resected from a venous segment of an overflow AV fistula from 6 patients and from a normal cephalic vein from 4 other patients. The surgical specimens were immediately placed in test tubes containing RNeater (see below for details).

Total RNA was extracted from the venous tissue samples, and gene expression between the 2 groups was compared using Whole Human Genome DNA microarray 44 K (Agilent Technologies, Santa Clara, California). The microarray data were analyzed with GeneSpring GX software and Ingenuity Pathway Analysis.

5. RNA Isolation

Surgical specimens were 0.5 cm or smaller in size and were initially stored in RNA later (Ambion, Austin, TX) overnight at 4 ± 3°C then at −80°C until RNA extraction. Total RNA was extracted using TRIZol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. The total RNA was further purified using the Qiagen RNeasy Mini Kit (Qiagen, Valencia, CA) and then extracted. The quantity and quality of the RNA were determined using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA) and an Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA).

6. cRNA Amplification and Labeling

Total RNA was amplified and labeled with Cyanine 3 (Cy3) as instructed by the manufacturer of the Agilent Low Input Quick Amp Labeling Kit, one-color (Agilent Technologies, Palo Alto, CA). Briefly, 100 ng of total RNA was reverse transcribed to double-strand cDNA using a poly dT-T7 promoter primer. The primer, template RNA, and quality-control transcripts of known concentration and quality were then denatured at 65°C for 10 min and incubated for 2 hours at 40°C with 5X First-Strand Buffer, 0.1 M DTT, 10 mM dNTP mix, and Affinity Script RNase Block Mix. The Affinity Script enzyme was inactivated at 70°C for 15 min. The resulting cDNA products were then used as templates for in vitro transcription to generate fluorescent cRNA. They were mixed with a transcription master mix in the presence of T7 RNA polymerase and Cy3-labeled CTP and incubated at 40°C for 2 hours. Labeled cRNAs were purified using Qiagen's RNeasy Mini spin columns and eluted in 30 μL of nuclease-free water. After amplification and labeling, cRNA quantity and cyanine incorporation were determined using a Nanodrop ND-1000 spectrophotometer and an Agilent Bioanalyzer.

7. Sample Hybridization

For each hybridization, 1.65 μg of Cy3-labeled cRNA was fragmented and hybridized onto an Agilent Human GE 4x44K v2 Microarray (Design ID: 026652) for 17 hours at 65°C. After washing, the microarrays were scanned using an Agilent DNA microarray scanner.

8. Microarray Data Analysis

The intensity values of each scanned feature were quantified using Agilent feature extraction software (version 10.7.3.1), which performs background subtractions. We only used features flagged as having no errors (present flags) and excluded features that were not positive, not significant, not
Table 1: Patient characteristics.

| Over flow AVF | Age | Gender | Cause of CRF | Patency period of AV fistula (months) | Blood flow (mL/min) |
|---------------|-----|--------|--------------|---------------------------------------|--------------------|
| 1             | 48  | M      | CGN          | 56                                    | 3790               |
| 2             | 83  | F      | CGN          | 93                                    | 2760               |
| 3             | 57  | M      | CGN          | 19                                    | 3280               |
| 4             | 46  | M      | CGN          | 22                                    | 2710               |
| 5             | 75  | M      | CGN          | 104                                   | 3520               |
| 6             | 57  | F      | IgA          | 88                                    | 2340               |

Control

|                 |     |        |             | (−)                                   | (−)                |
|-----------------|-----|--------|-------------|---------------------------------------|--------------------|

Table 2: Genes significantly upregulated in the remodeled vein compared to the control vein (top 30).

| Probe name      | P value | FCAbsolute | Gene symbol |
|-----------------|---------|------------|-------------|
| A_23_P106194    | 0.045653246 | 38.85     | FOS         |
| A_23_P4299998   | 0.021514166 | 27.22     | FOSB        |
| A_24_P33895     | 0.001204797 | 25.26     | ATF3        |
| A_24_P46936     | 1.36E−05  | 24.77     | EGR2        |
| A_23_P46158     | 0.002943707 | 24.00     | KRT17       |
| A_23_P34915     | 0.001120758 | 23.39     | ATF3        |
| A_23_P71037     | 0.004629572 | 21.98     | IL6         |
| A_23_P46429     | 7.94E−04  | 21.04     | CYR61       |
| A_23_P882732    | 0.036205057 | 19.08     |             |
| A_23_P97141     | 0.0211732  | 17.66     | RGS1        |
| A_23_P323751    | 9.21E−05  | 17.26     | FAM83D      |
| A_33_P3316272   | 0.00369598  | 15.81     | MYOC        |
| A_23_P216225    | 0.00219925  | 15.72     | GPM6A       |
| A_33_P3295203   | 0.001008955 | 15.65     | HAS1        |
| A_23_P13208     | 2.92E−04  | 14.26     | NR4A2       |
| A_23_P214080    | 0.00224604  | 13.9      | EGR1        |
| A_33_P321408    | 7.03      | 13.40     | TRIM36      |
| A_23_P97141     | 0.0211732  | 12.66     | FAM83D      |
| A_33_P3316272   | 10.96     | 12.41     | CCL3        |
| A_24_P216225    | 0.00219925  | 12.41     | TRIM36      |
| A_33_P3365799   | 6.94      | 12.00     | NCAM1       |
| A_24_P214080    | 6.80      | 11.91     | DCAF12L1    |
| A_33_P3365799   | 6.94      | 11.91     | DCAF12L1    |
| A_33_P3365799   | 6.94      | 11.91     | DCAF12L1    |
| A_23_P13208     | 2.92E−04  | 11.26     | NR4A2       |
| A_23_P321408    | 7.03      | 11.00     | CCL3        |
| A_24_P71037     | 0.0211732  | 10.60     | RGS1        |
| A_23_P321408    | 7.03      | 10.60     | RGS1        |
| A_33_P3365799   | 6.94      | 10.60     | CCL3        |
| A_23_P13208     | 2.92E−04  | 10.60     | CCL3        |
| A_33_P321408    | 7.03      | 10.60     | CCL3        |
| A_23_P214080    | 7.03      | 10.60     | CCL3        |
| A_33_P3365799   | 6.94      | 10.60     | CCL3        |
| A_23_P13208     | 2.92E−04  | 10.60     | CCL3        |
| A_33_P321408    | 7.03      | 10.60     | CCL3        |
| A_23_P214080    | 7.03      | 10.60     | CCL3        |

Table 3: Genes significantly downregulated in the remodeled vein compared to the control vein (top 30).

| Probe name      | P value | FCAbsolute | Gene symbol |
|-----------------|---------|------------|-------------|
| A_23_P23783     | 18.99   | 0.009408315 | MYOC        |
| A_23_P121545    | 14.63   | 6.67E−04   | GPM6A       |
| A_33_P3368193   | 10.96   | 2.33E−05   | PNLP3PR3    |
| A_32_P92489     | 8.79    | 0.00495897 | PKD1L2      |
| A_24_P40626     | 8.15    | 0.01296479 | GREM2       |
| A_33_P3221408   | 8.14    | 0.00425862 | NTNG1       |
| A_23_P143526    | 7.15    | 0.00438317 | SITOB       |
| A_23_P136777    | 7.14    | 3.89E−04   | APOD        |
| A_23_P102331    | 7.10    | 0.00349091 | SC7A        |
| A_33_P34291923  | 7.03    | 0.00119926 | CADM3       |
| A_23_P40384     | 7.00    | 0.02654911 | CTSG        |
| A_33_P3363799   | 6.94    | 0.00268203 | NCAM1       |
| A_24_P203134    | 6.80    | 0.02463303 | DCIP2L1     |
| A_24_P280684    | 6.69    | 0.03005707 | FBXO40      |
| A_23_P55544     | 6.51    | 0.00426939 | CCB1        |
| A_23_P73571     | 6.45    | 0.03985146 | MUMI1       |
| A_23_P212050    | 6.22    | 0.02144801 | BCHE        |
| A_33_P3336557   | 6.12    | 1.20E−04   | TRIM36      |
| A_33_P3336557   | 6.12    | 1.20E−04   | TRIM36      |
| A_33_P3336557   | 6.12    | 1.20E−04   | TRIM36      |
| A_33_P3336557   | 6.12    | 1.20E−04   | TRIM36      |
| A_33_P3336557   | 6.12    | 1.20E−04   | TRIM36      |
| A_33_P3336557   | 6.12    | 1.20E−04   | TRIM36      |

uniform, not above background levels, saturated, or population outliers (marginal and absent flags). Normalization was performed using Agilent GeneSpring GX version 11.0.2. (per chip: normalization to the 75 percentile shift; per gene: normalization to median across all samples). There are 34,127 probes in total on the Agilent Human GE 4x44K v2
| GO accession (with AmiGO link) | GO term                                           | Biological process                                                                 | Corrected P value | Count in selection | % count in selection | Count in total | % count in total |
|--------------------------------|--------------------------------------------------|-------------------------------------------------------------------------------------|-------------------|---------------------|---------------------|---------------|------------------|
| GO:00032502                   | Developmental process                             |                                                                                    | 2.100E – 11       | 77                  | 29.8                | 3077          | 17.9             |
| GO:00007275                   | Multicellular organinal development               |                                                                                    | 1.340E – 10       | 67                  | 26.0                | 2810          | 16.3             |
| GO:0010033                    | Response to organic substance                     |                                                                                    | 3.530E – 10       | 40                  | 15.5                | 698           | 4.1              |
| GO:0001568                    | Blood vessel development                          |                                                                                    | 9.230E – 10       | 21                  | 8.1                 | 231           | 1.3              |
| GO:0048514                    | Blood vessel morphogenesis                        |                                                                                    | 1.250E – 09       | 19                  | 7.4                 | 198           | 1.1              |
| GO:0009144                    | Vasculature development                           |                                                                                    | 1.250E – 09       | 21                  | 8.1                 | 238           | 1.4              |
| GO:0048545                    | Response to steroid hormone stimulus              |                                                                                    | 1.360E – 09       | 23                  | 8.9                 | 183           | 1.1              |
| GO:001525                     | Angiogenesis                                      |                                                                                    | 2.930E – 09       | 18                  | 7.0                 | 139           | 0.8              |
| GO:0009653                    | Anatomical structure morphogenesis               |                                                                                    | 4.930E – 09       | 30                  | 11.6               | 1125          | 6.5              |
| GO:0016265                    | Death                                             |                                                                                    | 5.110E – 09       | 39                  | 15.1               | 663           | 3.8              |
| GO:0048646                    | Anatomical structure formation involved in morphogenesis |                                                                        | 5.950E – 09       | 18                  | 7.0                 | 306           | 1.8              |
| GO:0008219                    | Cell death                                        |                                                                                    | 1.280E – 08       | 37                  | 14.3               | 658           | 3.8              |
| GO:0042221                    | Response to chemical stimulus                     |                                                                                    | 1.280E – 08       | 53                  | 20.5               | 1264          | 7.3              |
| GO:0048856                    | Anatomical structure development                  |                                                                                    | 1.320E – 08       | 42                  | 16.3               | 2437          | 14.1             |
| GO:0006950                    | Response to stress                                |                                                                                    | 2.080E – 08       | 51                  | 19.8               | 1642          | 9.5              |
| GO:0048731                    | System development                                |                                                                                    | 3.600E – 08       | 38                  | 14.7               | 2284          | 13.3             |
| GO:0032570                    | Response to progesterone stimulus                 |                                                                                    | 7.370E – 08       | 9                   | 3.5                | 21            | 0.1              |
| GO:0006915|GO:0008632                           | Apoptosis                                        |                                                                                    | 1.340E – 07       | 31                  | 12.0               | 541           | 3.1              |
| GO:0012501|GO:0016244                           | Programmed cell death                           |                                                                                    | 1.900E – 07       | 31                  | 12.0               | 549           | 3.2              |
| GO:0042981                    | Regulation of apoptosis                           |                                                                                    | 2.470E – 07       | 35                  | 13.6               | 796           | 4.6              |
| GO:0032501|GO:0050874                           | Multicellular organinal process                  |                                                                                    | 3.190E – 07       | 70                  | 27.1               | 4154          | 24.1             |
| GO:0043067|GO:0043070                           | Regulation of programmed cell death              |                                                                                    | 3.190E – 07       | 35                  | 13.6               | 804           | 4.7              |
| GO:0010941                    | Regulation of cell death                         |                                                                                    | 3.310E – 07       | 35                  | 13.6               | 807           | 4.7              |
| GO:0009887                    | Organ morphogenesis                               |                                                                                    | 3.510E – 07       | 26                  | 10.1               | 685           | 4.0              |
| GO:0009628                    | Response to abiotic stimulus                      |                                                                                    | 5.270E – 07       | 16                  | 6.2                | 357           | 2.1              |
| GO:0009725                    | Response to hormone stimulus                      |                                                                                    | 5.390E – 07       | 23                  | 8.9                | 358           | 2.1              |
| GO:0048519|GO:0043118                           | Negative regulation of biological process        |                                                                                    | 6.290E – 07       | 39                  | 15.1               | 1756          | 10.2             |
| GO:0009719                    | Response to endogenous stimulus                   |                                                                                    | 7.450E – 07       | 24                  | 9.3                | 391           | 2.3              |
| GO:0009605                    | Response to external stimulus                     |                                                                                    | 7.730E – 07       | 32                  | 12.4               | 869           | 5.0              |
| GO:0048513                    | Organ development                                 |                                                                                    | 1.510E – 06       | 34                  | 13.2               | 1682          | 9.8              |
| GO:0009607                    | Response to biotic stimulus                       |                                                                                    | 2.160E – 06       | 23                  | 8.9                | 385           | 2.2              |
| GO:0070482                    | Response to oxygen levels                         |                                                                                    | 3.460E – 06       | 14                  | 5.4                | 137           | 0.8              |
| GO:0009266                    | Response to temperature stimulus                  |                                                                                    | 3.950E – 06       | 9                   | 3.5                | 86            | 0.5              |
| GO:0050896|GO:0051869                           | Response to stimulus                             |                                                                                    | 6.040E – 06       | 89                  | 34.5               | 3356          | 19.5             |
| GO:0048523|GO:0051243                           | Negative regulation of cellular process          |                                                                                    | 8.220E – 06       | 38                  | 14.7               | 1606          | 9.3              |
| GO:0009408|GO:0006951                           | Response to heat                                  |                                                                                    | 8.220E – 06       | 9                   | 3.5                | 61            | 0.4              |
| GO:0006928                    | Cellular component movement                       |                                                                                    | 1.200E – 05       | 14                  | 5.4                | 450           | 2.6              |
| GO:0050793                    | Regulation of developmental process               |                                                                                    | 1.590E – 05       | 8                   | 3.1                | 670           | 3.9              |
| GO:0048869                    | Cellular developmental process                    |                                                                                    | 1.600E – 05       | 24                  | 9.3                | 1641          | 9.5              |
| GO:0022603                    | Regulation of anatomical structure morphogenesis  |                                                                                    | 2.530E – 05       | 5                   | 1.9                | 228           | 1.3              |
| GO:0051239                    | Regulation of multicellular organinal process     |                                                                                    | 2.860E – 05       | 7                   | 2.7                | 924           | 5.4              |
| GO:0007565                    | Female pregnancy                                  |                                                                                    | 3.100E – 05       | 13                  | 5.0                | 104           | 0.6              |
| GO:0030454                    | Cell differentiation                              |                                                                                    | 4.450E – 05       | 24                  | 9.3                | 1576          | 9.1              |
| GO:0042127                    | Regulation of cell proliferation                  |                                                                                    | 4.830E – 05       | 29                  | 11.2               | 773           | 4.5              |
### Table 4: Continued.

| GO accession (with AmiGO link) | GO term                                      | Biological process | Corrected \( P \) value | Count in selection | % count in selection | Count in total | % count in total |
|-------------------------------|-----------------------------------------------|--------------------|---------------------------|-------------------|---------------------|----------------|-----------------|
| GO:0001666                    | Response to hypoxia                           |                    | \( 6.420 \times 10^{-5} \) | 14                | 5.4                 | 131           | 0.8             |
| GO:0008284                    | Positive regulation of cell proliferation     |                    | \( 7.720 \times 10^{-5} \) | 16                | 6.2                 | 410           | 2.4             |
| GO:0048522|GO:0051242 | Positive regulation of cellular process       | \( 7.720 \times 10^{-5} \) | 34                | 13.2                | 1806          | 10.5            |
| GO:0051789                    | Response to protein stimulus                  |                    | \( 8.360 \times 10^{-5} \) | 11                | 4.3                 | 96            | 0.6             |
| GO:0048518|GO:0043119 | Positive regulation of biological process     | \( 9.260 \times 10^{-5} \) | 35                | 13.6                | 1982          | 11.5            |
| GO:0043627                    | Response to estrogen stimulus                 |                    | \( 1.000 \times 10^{-4} \) | 12                | 4.7                 | 98            | 0.6             |
| GO:0009991                    | Response to extracellular stimulus            |                    | \( 1.000 \times 10^{-4} \) | 6                 | 2.3                 | 204           | 1.2             |
| GO:0042493|GO:0017035 | Response to drug                              | \( 1.770 \times 10^{-4} \) | 17                | 6.6                 | 213           | 1.2             |
| GO:0043066                    | Negative regulation of apoptosis              |                    | \( 1.790 \times 10^{-4} \) | 19                | 7.4                 | 345           | 2.0             |
| GO:0043069|GO:0043072 | Negative regulation of programmed cell death  | \( 2.200 \times 10^{-4} \) | 19                | 7.4                 | 350           | 2.0             |
| GO:0051384                    | Response to glucocorticoid stimulus           |                    | \( 2.200 \times 10^{-4} \) | 10                | 3.9                 | 70            | 0.4             |
| GO:0050798|GO:0050791 | Regulation of biological process              | \( 2.440 \times 10^{-4} \) | 109               | 42.2                | 7200          | 41.8            |
| GO:0060548                    | Negative regulation of cell death            |                    | \( 2.570 \times 10^{-4} \) | 19                | 7.4                 | 354           | 2.1             |
| GO:0051707|GO:0009613|GO:0042828 | Response to other organism                    | \( 2.770 \times 10^{-4} \) | 17                | 6.6                 | 300           | 1.7             |
| GO:0040011                    | Locomotion                                    |                    | \( 2.800 \times 10^{-4} \) | 16                | 6.2                 | 415           | 2.4             |
| GO:0009611|GO:0002245 | Response to wounding                          | \( 2.800 \times 10^{-4} \) | 21                | 8.1                 | 507           | 2.9             |
| GO:0031960                    | Response to corticosteroid stimulus           |                    | \( 3.860 \times 10^{-4} \) | 10                | 3.9                 | 75            | 0.4             |
| GO:0050794|GO:0051244 | Regulation of cellular process                | \( 4.070 \times 10^{-4} \) | 108               | 41.9                | 6938          | 40.3            |
| GO:0014070                    | Response to organic cyclic substance          |                    | \( 4.200 \times 10^{-4} \) | 12                | 4.7                 | 114           | 0.7             |
| GO:0051128                    | Regulation of cellular component organization |                    | \( 5.900 \times 10^{-4} \) | 6                 | 2.3                 | 466           | 2.7             |
| GO:0065007                    | Biological regulation                          |                    | \( 7.620 \times 10^{-4} \) | 109               | 42.2                | 7592          | 44.1            |
| GO:0051704|GO:0051706 | Multiorganism process                        | \( 7.900 \times 10^{-4} \) | 27                | 10.5                | 706           | 4.1             |
| GO:0031099                    | Regeneration                                   |                    | \( 8.770 \times 10^{-4} \) | 6                 | 2.3                 | 65            | 0.4             |
| GO:0007610                    | Behavior                                       |                    | \( 9.680 \times 10^{-4} \) | 12                | 4.7                 | 449           | 2.6             |

### Table 5: Statistically overrepresented GO terms in the molecular function category \((P < 0.01)\).

| GO accession (with AmiGO link) | GO term                                 | Molecular function | Corrected \( P \) value | Count in selection | % count in selection | Count in total | % count in total |
|-------------------------------|-----------------------------------------|--------------------|---------------------------|-------------------|---------------------|----------------|-----------------|
| GO:0005539                    | Glycosaminoglycan binding                |                    | \( 9.960 \times 10^{-6} \) | 14                | 5.4                 | 149           | 0.9             |
| GO:0005515|GO:0045308 | Protein binding                          | \( 1.550 \times 10^{-5} \) | 170               | 65.9                | 8104          | 47.0            |
| GO:0001871                    | Pattern binding                          |                    | \( 3.120 \times 10^{-5} \) | 14                | 5.4                 | 164           | 1.0             |
| GO:0030247                    | Polysaccharide binding                   |                    | \( 3.120 \times 10^{-5} \) | 14                | 5.4                 | 164           | 1.0             |
| GO:0008201                    | Heparin binding                          |                    | \( 6.710 \times 10^{-5} \) | 13                | 5.0                 | 112           | 0.7             |
| GO:0005126                    | Cytokine receptor binding                |                    | \( 7.930 \times 10^{-5} \) | 4                 | 1.6                 | 177           | 1.0             |
| GO:0005125                    | Cytokine activity                        |                    | \( 2.200 \times 10^{-4} \) | 12                | 4.7                 | 193           | 1.1             |
| GO:0005114                    | Type II transforming growth factor beta receptor binding | | \( 1.143 \times 10^{-3} \) | 4                 | 1.6                 | 7             | 0.0             |
| GO:0008083                    | Growth factor activity                   |                    | \( 2.365 \times 10^{-3} \) | 13                | 5.0                 | 160           | 0.9             |
| GO:0005102                    | Receptor binding                         |                    | \( 3.106 \times 10^{-3} \) | 24                | 9.3                 | 873           | 5.1             |
| GO:0030246                    | Carbohydrate binding                     |                    | \( 7.049 \times 10^{-3} \) | 14                | 5.4                 | 354           | 2.1             |
Table 6: Statistically overrepresented GO terms in the cellular component category (P < 0.01).

| GO accession (with AmiGO link) | GO term                                      | Corrected P value | Count in selection | % count in selection | Count in total | % count in total |
|--------------------------------|----------------------------------------------|-------------------|--------------------|----------------------|----------------|------------------|
| GO:0044421 Extracellular region part | 2.190E−09     | 49        | 19.0               | 937                  | 5.4            |                  |
| GO:0030102 Extracellular matrix  | 7.420E−07     | 25        | 9.7                | 339                  | 2.0            |                  |
| GO:0005576 Extracellular region  | 3.880E−06     | 69        | 26.7               | 1923                 | 11.2           |                  |
| GO:0005615 Extracellular space   | 1.690E−05     | 32        | 12.4               | 673                  | 3.9            |                  |
| GO:0005578 Proteinaceous extracellular matrix | 1.200E−04 | 20        | 7.8                | 309                  | 1.8            |                  |
| GO:0060205 Cytoplasmic membrane-bounded vesicle lumen | 4.070E−04 | 7         | 2.7                | 44                   | 0.3            |                  |
| GO:0031983 Vesicle lumen          | 5.660E−04     | 7         | 2.7                | 46                   | 0.3            |                  |
| GO:0031093 Platelet alpha granule lumen | 2.468E−03 | 7         | 2.7                | 41                   | 0.2            |                  |

Microarray (Design ID: 026652), excluding control probes. The microarray data were submitted to NCBI GEO (http://www.ncbi.nlm.nih.gov/geo/), sample number [GSE39488].

The altered transcripts were quantified using a comparative method. We applied a P value < 0.05 combined with a >2-fold change in normalized intensity to identify genes with significantly different expression patterns.

9. Gene Ontology Analysis and Pathway Analysis

The gene ontology analysis was performed using Agilent Technologies GeneSpring GX software (11.0.2). Pathway analysis was performed with GenMAPP 2.1 (http://www.genmapp.org/).

10. Results

The cDNA microarray analysis revealed that 397 genes were upregulated and 456 were downregulated (Tables 2 and 3).

The gene ontology analysis revealed that biological developmental processes and glycosaminoglycan binding were the most upregulated. In addition, most upregulation occurred extracellularly (Tables 4, 5, and 6).

The pathway analysis revealed that the TGF beta signaling pathway, cytokines and inflammatory response pathway, hypertrophy model, and the myometrial relaxation and contraction pathway were upregulated (Table 7).

11. Discussion

AV fistulas are very useful for determining the optimal blood flow for hemodialysis since satisfactory blood access flow is necessary for adequate hemodialysis. When stenotic lesions occur within the vascular system and blood flow is insufficient, a percutaneous transluminal angioplasty or some other intervention is performed. However, overflow AV fistulas increase cardiac output and cause high-output cardiac failure [1].

In the 2005 Japanese Society for Dialysis Therapy Guidelines for Vascular Access Construction and Repair for Chronic Hemodialysis, vascular access flow is said to lead to heart failure when the blood access flow is greater than 1.0−1.5 L/min or when the vascular access flow/cardiac output ratio is >20% [1]. If the vascular access flow is clearly responsible for a decline in cardiac function, then it is necessary to intentionally constrict or occlude the vascular access [1]. Surveillance of blood flow in internal shunts by the Doppler echocardiography has become widespread and overflow AV fistulas are now actively treated. Several recent studies have noted the importance of histological changes in AV fistulas [4, 5].

Microarrays of vascular access have been reported in experimental animal models, but there have been no such analyses in humans [6]. In the present study, venous tissue samples were resected from overflow AV fistulas from 6 hemodialysis patients and from the normal cephalic veins of 4 other hemodialysis patients, and their gene expression patterns were compared.

It is interesting to note that zinc finger-containing transcription factors such as egr1, egr2, and egr3 and immediate early genes such as fos and jun, were found to be remarkably upregulated in the present study; egr1, egr 2, and egr 3 have been implicated in the proliferation and differentiation of many cell types [7, 8], and fos and jun have been linked to the regulation of angiogenesis [9]. Moreover, egr-1, c-jun, and c-fos have been linked to the regulation of free radical scavenging enzymes [10–13]. We also observed the upregulation of free radical scavenging enzyme activity in the walls of the overflow AV fistulas, which may reflect chronic reactive oxygen species formation in overflow AV fistulas.

The pathway analysis indicated that the TGF beta signaling pathway and cytokines and inflammatory response pathway were upregulated. This suggests that overflow AV fistulas may be implicated in chronic inflammation in hemodialysis patients.

Malnutrition, inflammation, and atherosclerosis (MIA syndrome) are common in end-stage renal disease (ESRD) patients, and inflammation has been identified as playing a key role in atherosclerotic cardiovascular disease. Proinflammatory cytokines are pivotal to the inflammation that is,
Table 7: Pathway analysis results.

| Pathway name                                      | LS_vs_control |
|--------------------------------------------------|---------------|
| Alpha6 beta4 integrin signaling pathway           | 0.793         |
| Androgen receptor signaling pathway               | 0.528         |
| Apoptosis mechanisms                              | 0.124         |
| B-cell receptor signaling pathway                 | 0.023         |
| G1 to S cell cycle control                        | 1             |
| Cell cycle                                        | 0.487         |
| Delta-Notch signaling pathway                     | 0.226         |
| DNA replication                                   | 1             |
| EGFR1 signaling pathway                           | 0.856         |
| FAS pathway and stress induction of HSP regulation| 1             |
| **Focal Adhesion**                                | **0.003**     |
| G13 signaling pathway                             | 0.269         |
| G protein signaling pathways                      | 0.258         |
| Hedgehog signaling pathway                        | 1             |
| Apoptosis modulation by HSP70                     | 1             |
| Id signaling pathway                              | 1             |
| IL-1 signaling pathway                            | 1             |
| IL-2 signaling pathway                            | 0.327         |
| IL-3 signaling pathway                            | 0.371         |
| IL-4 signaling pathway                            | 0.589         |
| IL-5 signaling pathway                            | 0.576         |
| IL-6 signaling pathway                            | 1             |
| IL-7 signaling pathway                            | 0.498         |
| IL-9 signaling pathway                            | 1             |
| Human insulin signaling                           | 0.387         |
| Integrin-mediated cell adhesion                   | 0.363         |
| Kit receptor signaling pathway                    | 0.051         |
| MAPK cascade                                      | 1             |
| MAPK signaling pathway                            | 0.011         |
| mRNA processing (Homo sapiens)                    | 0.014         |
| Notch signaling pathway                           | 0.191         |
| Ovarian infertility genes                         | 1             |
| p38 MAPK signaling pathway                        | 0.108         |
| Regulation of actin cytoskeleton                  | 0.834         |
| Eukaryotic transcription initiation               | 0.511         |
| Signal transduction of SIP                        | 0.384         |
| Signaling of hepatocyte growth factor receptor    | 1             |
| T cell receptor signaling pathway                 | 0.243         |
| TGF-beta receptor signaling pathway               | 0.095         |
| **TGF beta signaling pathway**                    | **0.00**      |
| TNF-alpha/NF-xB signaling pathway                 | 0.752         |
| Translation factors                               | 0.368         |
| Wnt signaling pathway                             | 0.15          |
| Wnt signaling pathway                             | 0.051         |
| Acetylcholine synthesis                           | 1             |
| Alanine and aspartate metabolism                  | —             |
| Biogenic amine synthesis                          | 1             |
Table 7: Continued.

| Pathway name                                      | LS vs control |
|---------------------------------------------------|---------------|
| Cytokines and inflammatory response (BioCarta)    | 0             |
| Hypertrophy model                                 | 0             |
| Inflammatory response pathway                     | 0.054         |
| Irinotecan pathway (Homo sapiens)                 | 0.685         |
| Oxidative stress                                  | 0.402         |
| Proteasome degradation                            | 0.278         |
| Myometrial relaxation and contraction pathways     | 0             |
| Striated muscle contraction                       | 0.427         |

associated with malnutrition and atherosclerosis in ESRD [14]. Our findings suggest that overflow AV fistulas may be implicated in MIA syndrome.

12. Conclusion

Combining microarray results and pathway information available via the Internet provided biological insight into molecular changes in the venous walls of overflow AV fistulas. Despite the small sample size, our study findings suggest that overflow AV fistulas may be implicated in chronic inflammation in hemodialysis patients.

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