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Differential Gene Expression In Coiled Versus Flow Diverter-Treated Aneurysms: RNA Sequencing Analysis In Rabbit Aneurysm Model

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Abstract

Introduction—The biological mechanisms leading to aneurysm healing or rare complications such as delayed aneurysm ruptures after flow-diverter placement remain poorly understood. We used RNA-sequencing (RNA-seq) following implantation of coils or flow-diverters in elastase aneurysms in rabbits to identify genes and pathways of potential interest.

Methods—Aneurysms were treated with coils (n=5) or flow-diverters (n=4) or left untreated for controls (n=6). Messenger RNA were isolated from the aneurysms at 4 weeks following treatment. RNA samples were processed using RNA-seq technology and analyzed using the Ingenuity Pathway Analysis tool.

Results—Using RNA-seq for coiled versus untreated aneurysms, 464/9990 genes (4.6%) were differentially expressed (58 down-regulated, 406 up-regulated). Comparing flow-diverter versus untreated aneurysms, 177/10041 (1.8%) genes were differentially expressed (8 down-regulated, 169 up-regulated). Comparing flow-diverter versus coiled aneurysms, 13/9982 (0.13%) genes were differentially expressed (8 down-regulated, 5 up-regulated). Keratin 8 was overexpressed in flow-diverters versus coils. This molecule may potentially play a critical role in delayed ruptures due to plasmin production. We identified overregulation of apelin in flow-diverters supporting the preponderance of endothelialization, whereas we found overexpression of molecules implicated in wound healing (Dectin1 and HHIP) for coiled aneurysms. Furthermore, we identified metallopeptidases 1, 12 and 13 as overexpressed in coiled versus untreated aneurysms.

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Conclusions—We observed different physiopathologic responses after endovascular treatment with different devices. Flow-diverters promote endothelialization but express molecules that could potentially explain the rare delayed ruptures. Coils promote wound healing and express genes potentially implicated in recurrence of coiled aneurysms.

INTRODUCTION

Endovascular treatment is now considered standard of care for the treatment of most intracranial aneurysms (IA). Numerous endovascular tools exist for the treatment of IA and flow-diverting devices have gained a large interest with good occlusion rates[1]. However, the biological mechanisms driving IA physiopathology remain poorly understood, including the mechanisms for formation, rupture, growth, healing or device-related complications need of further elucidation. Indeed, endovascular devices used for the treatment of IAs are not simply inert mechanical devices used to seal the aneurysm neck without any interaction with the host, rather, they interact with different biological processes with the aim to definitely heal the aneurysm. Those biological interactions may vary according to the device used or depending on the local biological conditions and sometimes lead to non-occlusion of the aneurysm or to very rare but devastating complications such as delayed rupture[2–4]. It is of high importance to understand biological processes after endovascular treatment in order to optimize the devices used for the treatment of IA and try to prevent potential complications.

Previous studies aimed at exploring the mechanisms of aneurysm healing following endovascular treatments but have mostly focused at the tissue, cellular or molecular levels[5–7]. Endovascular coiling primarily elicits thrombus formation in the aneurysm cavity and then promotes neointima formation across the neck to seal the aneurysm cavity from the circulation[5, 8], but long term occlusion rates are poor with high rates of recanalization due to lack of aneurysms healing[9, 10]. On the contrary, occlusions rates following flow diverters are high and likely driven by endothelialization of the device from endothelial cells originating from the parent artery[6, 11]. However, despite high rates of occlusion and good clinical outcomes[5], flow-diverter devices have been associated with the occurrence of previously unobserved complications. Indeed, several cases of delayed aneurysm ruptures have been reported with fatal outcomes[3, 4]. Even if this complication is very rare and occurs in lees than 1% of cases, controversy exists surrounding their mechanisms and it appears important to try to explain it. Several mechanisms have been proposed to explain this complication, such as flow modifications[2] or a deleterious impact of the intra-aneurysms thrombus trapped by the flow-diverter[3]. Gene regulation studies have previously investigated the impact of selected key molecules such as metallopeptidases, fibronectin and collagen, potentially involved in the healing of aneurysms following coil or flow diverter embolization[12–14]. However, these prior studies did not provide a global overview of the biological pathways involved in those different treatment options[15]. Recently, microarray and RNA-sequencing (RNA-seq) have been used to compare mRNA and miRNA expression either in both humans and animal models in order to better understanding the molecular mechanisms of aneurysm healing[16, 17]. However, none of these previous studies have compared coiled or flow-diverter treated aneurysms[18]. We used RNA-seq technology following implantation of coils or flow-diverters in elastase induced saccular aneurysms in rabbits to identify genes and pathways of

potential clinical interest and to determine if differential pathways exist for healing of coiled and flow-diverter treated aneurysms.

MATERIALS AND METHODS

Aneurysm Creation, Treatment, and Follow-up

The Institutional Animal Care and Use Committee approved all procedures before initiation of the study. Some of the rabbits employed in this study were originally used as part of another investigations, where we compared the gene expression between untreated aneurysms with contralateral carotid arteries[16] and in prior analyses of expression of selected vascular remodeling molecules following coil and flow diverter treatment[15]. Elastase induced saccular aneurysms were created in 16 New Zealand White rabbits (body weight 3–4 kg). Detailed procedures for aneurysm creation have been previously described in depth[19]. Aneurysms were permitted to mature for at least 3 weeks after creation. Then aneurysms were either embolized with platinum coils (n=5), or treated with flow diverters (Pipeline Embolic Device, Covidien Inc, California, USA) as previously described[20] (n=4) or left untreated (n=6). At 4 weeks following treatment, a follow-up DSA of the aortic arch was performed. The animals were then euthanized using a lethal injection of pentobarbital. Untreated aneurysms were euthanized at 12 weeks following aneurysm creation. The aneurysm samples were harvested and the samples were immediately snap frozen in liquid nitrogen and kept frozen at −70°C until use.

RNA Extraction

RNA was isolated from frozen tissues by using miRNeasy Mini Kit (Qiagen, Calif). The quantity of the RNA was measured by using spectrophotometry, and the integrity of the RNA was confirmed by electrophoretic separation by using the 2100 Bioanalyzer (Agilent Technologies, Calif).

RNA Sequencing

RNA libraries were prepared according to the manufacturer’s instructions for the TruSeq RNA Sample Prep Kit v2 (Illumina, CA). Then the libraries were loaded onto paired end flow cells following Illumina’s standard protocol using the Illumina cBot and cBot Paired end cluster kit version 3 and HCS v2.0.12 data collection software. Base calling were performed using Illumina’s RTA version 1.17.21.3.

Bioinformatics Analysis

The processing of the mRNA and miRNA data was performed using MAP-RSeq (v1.2.1.3)[21]. MAPRSeq consists of the following steps: alignment, quality control, obtaining genomic features per sample and finally summarizing the data across samples. The pipeline provides detailed quality control data to estimate the distance between paired-end reads, evaluates the sequencing depth for alternate splicing analysis, determines the rate of duplicate reads, and calculates the read depth across genes using the RSeQC (v2.3.2)[22] software. Paired-end reads are aligned by TopHat (v2.0.6)[23] against the April 2009 oryCun2 genome build using the bowtie1[24] aligner option. Gene counts were generated using HTSeq (v0.5.3p9)[25] software and the gene annotation files were obtained from Ensembl (ftp://

ftp.ensembl.org/pub/release75/gtf/oryctolagus_cuniculus/Oryctolagus_cuniculus.OryCun2.0.75.gtf.gz) and the University of California Santa Cruz (http://hgdownload.soe.ucsc.edu/downloads.html#rabbit). Differential expression comparing a sample's normal tissue to that same sample's tissue with an aneurysm was computed using the edgeR algorithm (v2.6.2) across all samples. Human orthologs were assigned using Exolocator[26]. The pathway analysis leveraged the Ingenuity Pathway Analysis (IPA)[27] software to identify pathways enriched with human ortholog targets. IPA identified the involvement of different pathways according to the number of genes of the specific pathway which were differentially expressed in the compared groups. A pathway is considered more involved than an other if a larger amount of genes of this specific pathway are found up or down-regulated according to the pre-specified values.

Quantitative real time PCR analysis

The mRNA expression of selected genes was assessed by quantitative real time PCR. These selected genes were prion protein 2 (PRND), fibroblast growth factor 23 (FGF-23), matrix metalloproteinase (MMP) 1, SRC kinase signaling inhibitor 1 (SRCIN1), death associated protein-like 1 (DAPL1) and hedgehog-interacting protein (HHIP). Briefly, first strand complementary DNAs were synthesized from 500 ng of total RNA using a synthesis system (SuperScript III First-Strand Synthesis System; Invitrogen, USA). Then real time PCR was performed with a cycler (iCycler; Bio-Rad, USA) using SYBR Green PCR kit (Invitrogen). The specific primers were designed from corresponding sequences obtained from GenBank using a Web tool (Primer 3; http://frodo.wi.mit.edu/primer3/).

Statistical Analysis

The t-test statistics and corresponding P values were used as a measure of the mean change in expression between the test and control groups relative to the variability. The primary assessment was comparing each treatment group versus the control group. We additionally had a secondary assessment comparing treatment groups against each other. EdgeR tool was used to test for a normal distribution of the data. The t test based P values were adjusted for multiple comparisons by using the false discovery rate (FDR) multiple correction approach[28]. Genes were considered differentially expressed in case of a fold change of 2 (log value > 2 were considered up regulated, whereas log value < −2 were considered down regulated), with a FDR ≤ 0.1 and a P value < 0.05.

RESULTS

Coiled versus untreated aneurysms

All aneurysms treatments with coils were successful without any recurrence at follow-up. Using the criteria above for differential expression, 464 out of 9990 (4.6%) genes were identified as being differentially expressed when compared to untreated aneurysms. Of these 464 genes, 58 were down-regulated and 406 were up-regulated (Online table 1). The most up and down regulated molecules are presented in Table 1. Of the 10 most up-regulated, 3 are metalloproteinases: MMP1 (8.4 fold), MMP12 (6.1 fold) and MMP13 (7.2 fold) involved in the breakdown of extracellular matrix and interstitial collagen for tissue remodeling. The most down-regulated protein is HHIP, decreased 3.5 fold compared to untreated aneurysms.

The most involved pathways are shown in online table 2. Those pathways are generally related to inflammatory responses, including T and B cells and interleukin-10 involvement and cell-to-cell signaling as well as granulocytes and agranulocytes adhesion and diapedesis. Those pathways involve up-regulation of MMPs such as MMP1, MMP3, MMP12, MMP13 and interleukins.

Flow-diverter treated versus untreated aneurysms

All aneurysms treatments with FD were successful without any delayed rupture at follow-up. Using the criteria above for differential expression, 177 out of 10041 (1.8%) genes were identified as being differentially expressed. Of these 177 genes, 8 were down-regulated and 169 were up-regulated (Online table 3). The most up and down regulated molecules are presented in Table 2. Of the 10 most up-regulated, FGF23 is increased 5.7 fold, keratin 8 (KRT8) increased 6.2 fold, MMP1 increased 4.5 fold, apelin (APLN) increased 4.4 fold and interleukin 6 (IL6) increased 4.4 fold compared to untreated aneurysms. Of the most down-regulated molecules, DAPL1 is decreased by 3.8 fold, SRCIN1 is decreased by 3.3 fold, macrophage receptor with collagenous structure (MARCO) is decreased by 2.4 fold, Fibroblast growth factor binding protein 1 (FGFBP1) is decreased by 2.2 fold. The most involved pathways are shown in online table 4. The most involved pathway is the atherosclerosis signaling pathways with 9 up-regulated genes when compared to non-treated aneurysms. Similarly to coiled aneurysms, agranulocytes adhesion and diapedesis pathway, as well as cell-to-cell signaling pathway are involved with flow-diverters.

Flow-diverter treated versus coiled aneurysms

Using the criteria above for differential expression, 13 out of 9982 (0.13%) genes were identified as being differentially expressed. Of these 13 genes, 8 were down-regulated and 5 were up-regulated (Table 3). Of the 13 differentially expressed molecules when comparing flow-diverter treated IA to coiled, KRT8 was increased 4.3 fold, and basigin (BSG) was increased 3.8 fold. Protein disulfide isomerase-like (PDILT) was over-expressed in the coiled group compared to the flow-diverter group 4.2 fold and C-type lectin domain family 7, member A (CLEC7A, also called dectin 1) was over-expressed 2.5 fold in the coiled group. Due to the low number of differentially expressed molecules when comparing coiled with flow-diverter treated aneurysms, it was not possible to identify specific pathways differentially involved.

Validation of Microarray Data

Verification of differential gene expression in the aneurysm and control artery was done in five selected genes from the top up or down-regulated genes identified by RNA-seq. Those selected genes were PRND, FGF-23, MMP1, SRCIN1, DAPL1 and HHIP. Results obtained by RT-PCR for gene expression levels were varying in the same way and in comparable amplitude than did with RNA-seq. Results of RT-PCR are presented in online table 5.

DISCUSSION

This study found differential expression in a large assortment of genes in tissue from coiled or flow-diverter treated aneurysms compared with untreated aneurysms. The differentially

expressed genes are mostly related to the inflammatory response and cellular migration. These findings may provide insight into the biological effects of coils and flow diverters and also highlight pathways to analyze in order to better understand and optimize the outcomes after endovascular treatment for intracranial aneurysms.

Our results showed that relatively few genes were differentially expressed when comparing coils versus flow-diverter treated aneurysms. These findings highlight the fact that, despite two different approaches, the response to the device used for the endovascular treatment of IA does not vary substantially. These findings further demonstrate that the observed gene modifications were mostly driven by the aneurysm itself than by the device and implies that the mechanisms leading to aneurysm occlusion are somewhat similar, whatever the device used. However, some genes where differentially expressed in the flow-diverter treated group compared to the coiled group.

Specifically, we identified that the most up-regulated molecule is keratin 8, which acts as a binding site for plasminogen[29]. This overexpression of plasminogen receptors could be deleterious for the treated aneurysms. Indeed, association of plasminogen with cellular receptors facilitates its activation in plasmin[30, 31]. Then, plasmin generated from plasminogen is able to degrade extracellular matrix components directly or indirectly by activating MMPs (MMP-1, 3 and 9)[32, 33].

Our study also found that apelin is up-regulated in flow-diverter treated aneurysms compared to coiled aneurysms. This molecule significantly reduces aneurysm formation in the elastase model of AAA by decreasing macrophage burden likely due to an apelin-mediated decrease in proinflammatory cytokine and chemokine activation[34, 35]. It has also been demonstrated that apelin is present to a limited degree in endothelial cells, with a potent ability to stimulate the proliferation of cultured human umbilical vein endothelial cells[36]. In our study, apelin was overexpressed in flow-diverter treated aneurysms compared to coiled, which is potentially a key factor for the promotion of endothelial cells, leading to stent endothelialization and aneurysm occlusion[37].

These current results also confirm the role of inflammation in responding implanted devices for the treatment of IA. Metalloroteinases are known to be involved in thrombus homeostasis in IA but mainly MMP2 and MMP9 have been described in this pathology[38–41]. Here, we report the important role of other MMP molecules (MMP1, 12 and 13) over-expressed in coiled versus untreated IA. These MMPs have been reported as being implicated in abdominal aortic aneurysms (AAA) formation and progression[42–52]. However their impact has not been extensively descripted in the setting of IA[50]. We can suspect that this increased level of MMPs in coiled aneurysms is linked with recanalization as MMP-9 levels are associated with aneurysm recanalization and recurrence[53]. The RNA-seq also found that basigin (also known as extracellular matrix metalloproteinase inducer: EMMPRIN) is up-regulated in flow-diverter treated aneurysms compared to coiled aneurysms. This molecule is known to regulate different MMPs, especially MMP2 and MMP9[54, 55]. The increased level of basigin in flow-diverters could explain the higher level of those MMP in flow-diverter treated aneurysms as previously described[15].

Furthermore, the Macrophage Receptor with Collagenous Structure (MARCO) is another differentially expressed gene in our study. This molecule is associated with thrombus-free aneurysms in a study comparing thrombus-free and thrombus-covered wall of AAA[56]. In our study, we observed a down-regulation of MARCO in the flow-diverter group, suggesting an increased implication of intra-aneurysmal thrombosis compared to untreated aneurysms and the potential role of intra-aneurysmal thrombus for delayed aneurysm rupture associated with flow-diverters[3, 57].

Regarding the potential deleterious role of keratin 8 in flow-diverters, the generation of plasmin induces neutrophil aggregation, monocyte chemotaxis and expression of pro-inflammatory molecules[58] via multiple signaling pathways including nuclear factor-κB (NF-κB)[59]. This involvement of the fibrinolytic system has been previously highlighted in AAA pathology[60]. In AAA, it has been described that plasminogen is present in the mural thrombus[61]. This mural thrombus, by trapping polymorphonuclear leukocytes and adsorbing plasma components could act as a source of proteases in aneurysms that may play a critical role in enlargement and rupture[57]. Furthermore, AAA diameter is correlated with the level of plasmin activity in AAA wall[61]. The overexpression of keratin 8 in flow-diverter treated IA, could explain the deleterious issue in the rare cases of delayed aneurysms ruptures after FDs. This overexpression of keratin 8 associated with large amount of intra-saccular thrombus after flow-diverter placement, supports that intra-aneurysmal thrombosis is a possible cause of delayed aneurysm rupture after flow-diversion treatment[3], however the confirmation of this hypothesis would need further dedicated experiments to precise the impact of keratin 8.

Another important function for aneurysm occlusion after endovascular treatment is wound healing, consisting subsequently in thrombus formation, myofibroblast invasion, and extracellular matrix deposition[5, 12, 62]. CLEC7A (dectin 1) is a molecule promoting wound healing by the enhanced production of collagen matrices with beta-glucans[63–65]. Our results show that dectin 1 is about four times over-expressed in coiled IA when compared to flow-diverter treated IA. This suggests that wound healing is a process which is much more preponderant in coils than in flow-diverter. FGFBP1 is another molecule promoting wound healing[66, 67]. The present results show that FGFBP1 is decreased in flow diverter treated aneurysms compared to untreated aneurysms, supporting the idea that aneurysm occlusion after flow diverter therapy is not related to wound healing mechanisms but mostly by endothelial cells proliferation originating from the parent artery, as previously demonstrated[6]. We also identified another molecule of interest, HHIP, which is abundantly expressed in vascular endothelial cells and involved in angiogenesis[68]. We observed in our study that the expression of HHIP is down-regulated in coiled aneurysms. HHIP down-regulation is involved in the promotion of angiogenesis and could be involved in the neovascularization of the wound during the healing of coiled aneurysms[5, 69].

Limitations

Our study has several limitations. We used the rabbit elastase model and acknowledge that animal models are imperfect predictors of the human response. Indeed, the created aneurysms are in the mediastinum, rather than the subarachnoid space and thus subject to

different perianeurysmal modulations compared with berry aneurysms. Another limitation in using a model is the high degree of homogeneity among the different aneurysms, which is not the same in unselected human IA. However, this model has been evaluated with RNA-seq and has similar expression patterns to human intracranial aneurysms[16]. But this aneurysm model is not a model of spontaneous rupture and some biological pathways may differ when considering rupture-prone aneurysms. To explore these mechanisms, it could be interesting to analyze genes expressions in new models for active aneurysms with inflamed aneurysms wall or bio-active thrombus[70, 71]. Also, time intervals between creation of aneurysm and the time of euthanizing the animals were different between untreated and treated aneurysms which could introduce a difference in the healing process. Considering the differences between the human and the rabbit genomes, the observed findings may not be directly applicable to the clinical system. As with most gene expression studies, we recognize that any results obtained are exploratory in nature and need to be explored further; to that end, we did validate several results with RT–PCR and will continue to explore these results further in other models. Likewise, because of normal variations, there likely are genes for which our threshold levels were not achieved that may have an effect in humans. Just because a gene is not significantly up- or down-regulated, that does not necessarily imply that it is not relevant. Similarly, a gene found up or down-regulated is not necessarily related to the specific question. The aim of this study was to give a general overview of genes modifications after coiling or flow-diverter treatment; not to describe all the gene variations following coil embolization or flow-diverter therapy or to identify and focus on a specific pathways or molecules. This study identifies some new parameters to explore which could be potential key-factors to target to improve endovascular devices. This will require further validation with specific experiments to precisely describe the role of each molecule of interest.

CONCLUSION

RNA-sequencing analysis of rabbit aneurysms showed that despite different approaches, the response to the device used for the endovascular treatment of IA does not vary substantially and that the mechanisms leading to aneurysm occlusion are somewhat similar, whatever the device used. However, it revealed differential regulation of some key pathways, including inflammation and cellular migration that could explain the different biological mechanisms implicated in aneurysms healing either after coiling or flow-diverter treatments and could be key-molecules to explore in order to explain related complications. This study confirms that wound healing is preponderant after coiling compared to flow-diverter treated aneurysms. Also, this study identified in the flow-diverter treated IA an overexpreesion of Keratin 8 and Basigin, implicated in the inflammatory response and in the plasminogen system.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Top up and down-regulated molecules comparing coiled versus untreated aneurysms, determined by IPA.

Top up-regulated molecules		Exp. Value	Top down-regulated molecules		Exp. Value
MMP1	Matrix metallopeptidase 1 (interstitial collagenase)	**8.407**	**SRCIN1**	SRC kinase signaling inhibitor 1	**−4.210**
MAP3K19	Mitogen-activated protein kinase kinase kinase 19	**7.854**	**DAPL1**	Death associated protein-like 1	**−3.922**
TREML2*	Triggering receptor expressed on myeloid cells-like 2	**7.583**	**HHIP**	Hedgehog interacting protein	**−3.512**
FGF23	Fibroblast growth factor 23	**7.423**	**MPZ**	Myelin protein zero	**−3.400**
MMP13	Matrix metallopeptidase 13 (collagenase 3)	**7.201**	**MYH7B**	Myosin, heavy chain 7B, cardiac muscle, beta	**−3.342**
SLAMF7	SLAM family member 7	**6.670**	**PCDHAC2**	Protocadherin alpha subfamily C, 2	**−3.112**
CXCL13*	Chemokine (C-X-C motif) ligand 13	**6.490**	**THRSP**	Thyroid hormone responsive	**−3.089**
XDH	Xanthine dehydrogenase	**6.298**	**CYP2D6**	Cytochrome P450, family 2, subfamily D, polypeptide 6	**−2.991**
IBSP	Integrin-binding sialoprotein	**6.221**	**USH2A**	Usher syndrome 2A (autosomal recessive, mild)	**−2.932**
MMP12	Matrix metallopeptidase 12 (macrophage elastase)	**6.206**	**SORCS1**	Sortilin-related VPS10 domain containing receptor 1	**−2.703**

Values are expressed as log fold change.

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Table 2

Top up and down-regulated molecules comparing flow-diverted versus untreated aneurysms, determined by IPA.

Top up-regulated molecules		Exp. Value	Top down-regulated molecules		Exp. Value
PRND	Prion protein 2 (dublet)	**8.719**	**DAPL1**	Death associated protein-like 1	**−3.752**
KRT8	Keratin 8	**6.233**	**SRCIN1**	SRC kinase signaling inhibitor 1	**−3.295**
FGF23	Fibroblast growth factor 23	**5.662**	**FAM150A**	Family with sequence similarity 150, member A	**−2.555**
SIRPB1	Signal-regulatory protein beta 1	**5.463**	**MARCO**	Macrophage receptor with collagenous structure	**−2.396**
CXCL8*	Chemokine (C-X-C motif) ligand 8	**4.781**	**LECT1**	Leukocyte cell derived chemotaxin 1	**−2.373**
GPR158	G protein-coupled receptor 158	**4.747**	**SCIN**	Scinderin	**−2.287**
MMP1	Matrix metallopeptidase 1 (interstitial collagenase)	**4.467**	**FGFBP1**	Fibroblast growth factor binding protein 1	**−2.183**
HMGA2	High mobility group AT-hook 2	**4.412**	**MYH7B**	Myosin, heavy chain 7B, cardiac muscle, beta	**−2.107**
APLN	apelin	**4.412**			
IL6	Interleukin 6 (interferon, beta 2)	**4.375**			

Values are expressed as log fold change.

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Table 3

Top up and down-regulated molecules comparing flow-diverted versus coiled aneurysms, determined by IPA.

Top up-regulated molecules		Exp. Value	Top down-regulated molecules		Exp. Value
KRT8	Keratin 8	**4.266**	**PDILT**	Protein disulfide isomerase-like, testis expressed	**−4.172**
BSG	Basigin / EMMPRIN	**3.799**	**LIPA**	Lipase A, lysosomal acid, cholesterol esterase	**−3.820**
TLDC2	TBC/LysM-associated domain containing 2	**3.363**	**EXPH5**	Exophilin 5	**−2.872**
PRPH	Peripherin	**2.895**	**ZNF483**	Zinc finger protein 483	**−2.667**
RMRP	RNA component of mitochondrial RNA processing endoribonuclease	**2.671**	**KLHL14**	Kelch-like family member 14	**−2.470**
			CLEC7A	-type lectin domain family 7, member A	**−2.451**
			TCN1	Transcobalamin I (vitamin B12 binding protein, R binder family)	**−2.432**
			COL25A1	Collagen, type XXV, alpha 1	**−2.285**

Values are expressed as log fold change.