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Differential Gene Expression in Coiled versus Flow-Diverter-Treated Aneurysms: RNA Sequencing Analysis in a Rabbit Aneurysm Model

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ABSTRACT

BACKGROUND AND PURPOSE: The biologic mechanisms leading to aneurysm healing or rare complications such as delayed aneurysm ruptures after flow-diverter placement remain poorly understood. We used RNA sequencing following implantation of coils or flow diverters in elastase aneurysms in rabbits to identify genes and pathways of potential interest.

MATERIALS AND METHODS: Aneurysms were treated with coils ($n = 5$) or flow diverters ($n = 4$) or were left untreated for controls ($n = 6$). Messenger RNA was isolated from the aneurysms at 4 weeks following treatment. RNA samples were processed by using RNA-sequencing technology and were analyzed by using the Ingenuity Pathway Analysis tool.

RESULTS: With RNA sequencing for coiled versus untreated aneurysms, 464/9990 genes (4.6%) were differentially expressed (58 down-regulated, 406 up-regulated). When we compared flow-diverter versus untreated aneurysms, 177/10,041 (1.8%) genes were differentially expressed (8 down-regulated, 169 up-regulated). When we compared 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 (dectin 1 and hedgehog interacting protein) for coiled aneurysms. Furthermore, we identified metalloproteinases 1, 12, and 13 as overexpressed in coiled versus untreated aneurysms.

CONCLUSIONS: We observed different physiopathologic responses after endovascular treatment with various 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 the recurrence of coiled aneurysms.

ABBREVIATIONS: *DAPL1* = death associated protein-like 1; *FGFBP1* = fibroblast growth factor binding protein 1; *HHIP* = hedgehog-interacting protein; IA = intracranial aneurysm; *IL6* = interleukin 6; IPA = Ingenuity Pathway Analysis; *MMP* = matrix metalloproteinase; mRNA = messenger RNA; *PRND* = prion protein 2; RNA-seq = RNA sequencing; *SRCIN1* = SRC kinase signaling inhibitor 1

Endovascular treatment is now considered the standard of care for most intracranial aneurysms (IAs). Numerous endovascular tools exist for the treatment of IAs, and flow-diverting devices


have elicited much interest, with good occlusion rates.¹ However, the biologic mechanisms driving IA physiopathology remain poorly understood, including the mechanisms for formation, rupture, growth, healing, or device-related complications, and need 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 biologic processes with the aim of definitely healing the aneurysm. Those biologic interactions may vary according to the device used or depending on the local biologic conditions and sometimes lead to nonocclusion of the aneurysm or to very rare but devastating complications such as delayed rupture.²⁻⁴ It is important to understand the biologic processes after endovascular treatment to optimize the devices used for the treatment of IAs and try to prevent potential complications.


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Previous studies explored the mechanisms of aneurysm healing following endovascular treatments, but they 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 aneurysm healing.^{9,10} On the contrary, occlusions rates following flow diversion 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 rupture have been reported with fatal outcomes.^{3,4}

Even if this complication is very rare and occurs in <1% of cases, controversy exists surrounding the mechanism, and it appears important to try to explain it. Several mechanisms have been proposed to explain this complication, such as flow modifications² or a deleterious impact of the intra-aneurysm thrombus trapped by the flow diverter.³ Gene-regulation studies have previously investigated the impact of selected key molecules such as metalloproteinases, 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 biologic pathways involved in those different treatment options.¹⁵ Recently, microarray and RNA sequencing (RNA-seq) have been used to compare messenger RNA (mRNA) and microRNA expression in both humans and animal models to better understand 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 whether differential pathways exist for the healing of coiled and flow-diverter-treated aneurysms.

MATERIALS AND METHODS

Aneurysm Creation, Treatment, and Follow-Up

The Mayo Clinic Animal Care and Use Committee approved all procedures before initiation of the study. Some of the rabbits used in this study were originally used as part of another investigation, in which we compared the gene expression between untreated aneurysms with contralateral carotid arteries¹⁶ and prior analyses of the 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 they were either embolized with platinum coils ($n = 5$) or treated with flow diverters (Pipeline Embolization Device; Covidien, Irvine, California) as previously described²⁰ ($n = 4$) or left untreated ($n = 6$). At 4 weeks following treatment, follow-up DSA of the

aortic arch was performed. The animals were then euthanized by using a lethal injection of pentobarbital. Animals with 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 the miRNeasy Mini Kit (Qiagen, Valencia, California). 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, Santa Clara, California).

RNA Sequencing

RNA libraries were prepared according to the manufacturer's instructions for the TruSeq RNA Sample Prep Kit v2 (Illumina, San Diego, California). Then the libraries were loaded onto paired end-flow cells following the standard protocol of Illumina by using the cBot and cBot Paired-End Cluster Kit, Version 3, and HCS, Version 2.0.12 data collection software (Illumina). Base calling was performed by using RTA, Version 1.17.21.3 (Illumina).

Bioinformatics Analysis

The processing of the mRNA and microRNA data was performed by using MAP-RSeq (Version 1.2.1.3).²¹ MAP-RSeq 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 by using the RSeQC (Version 2.3.2)²² software. Paired-end reads are aligned by Tophat (Version 2.0.6; <https://ccb.jhu.edu/software/tophat/index.shtml>)²³ against the April 2009 OryCun2 genome build by using the Bowtie1.aligner²⁴ option (<http://www.broadinstitute.org/cancer/software/genepattern/modules/docs/Bowtie.aligner/4>). Gene counts were generated by using HTSeq (Version 0.5.3p9)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 the normal tissue versus aneurysm in the same rabbit was computed by using the edgeR algorithm (Version 2.6.2) across all samples. Human orthologs were assigned by using ExoLocator (<http://exolocator.bii.a-star.edu.sg/>).²⁶ The pathway analysis leveraged the Ingenuity Pathway Analysis (IPA; <http://www.winsite.com/ingenuity/ingenuity+pathway+analysis/>)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 that were differentially expressed in the compared groups. A pathway is considered more involved than another one if more genes of this specific pathway are found up- or down-regulated according to the prespecified values.

Table 1: Top up- and down-regulated molecules comparing coiled versus untreated aneurysms, determined by IPA^a

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

Note:—Exp. indicates expression.

^a Values are expressed as log-fold change.

Quantitative Real-Time Polymerase Chain Reaction Analysis

The mRNA expression of selected genes was assessed by quantitative real-time polymerase chain reaction. These selected genes were prion protein 2 (*PRND*), fibroblast growth factor-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 by using a synthesis system (SuperScript III First-Strand Synthesis System; Invitrogen, Grand Island, New York). Then, real-time polymerase chain reaction was performed with a cyclor (iCycler; Bio-Rad, Hercules, California) by using the SYBR Green PCR kit (Quantigen, Hilden, Germany). The specific primers were designed from corresponding sequences obtained from the GenBank by 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 compared each treatment group versus the control group. We additionally had a secondary assessment comparing treatment groups against each other. The 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 multiple-correction approach.²⁸ Genes were considered differentially expressed in case of a fold change of 2 (a log-value of >2 was considered up-regulated, whereas a log-value of ≤ 2 was considered down-regulated), with a false discovery rate ≤ 0.1 and a *P* value $< .05$.

RESULTS

Coiled versus Untreated Aneurysms

All aneurysm treatments with coils were successful without any recurrence at follow-up. Using the criteria above for differential expression, we identified 464 of 9990 (4.6%) genes as being dif-

ferentially expressed compared with untreated aneurysms. Of these 464 genes, 58 were down-regulated and 406 were up-regulated (On-line 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 with untreated aneurysms. The most involved pathways are shown in On-line Table 2. Those pathways are generally related to inflammatory responses, including T- and B-cell and interleukin-10 involvement and cell-to-cell signaling as well as granulocyte and agranulocyte 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 aneurysm treatments with flow diverters were successful without any delayed rupture at follow-up. Using the criteria above for differential expression, we identified 177 of 10,041 (1.8%) genes as being differentially expressed. Of these 177 genes, 8 were down-regulated and 169 were up-regulated (On-line Table 3). The most up- and down-regulated molecules are presented in Table 2. Of the 10 most up-regulated, fibroblast growth factor 23 increased 5.7-fold, keratin 8 increased 6.2-fold, *MMP1* increased 4.5-fold, apelin increased 4.4-fold, and interleukin 6 (*IL6*) increased 4.4-fold compared with untreated aneurysms. Of the most down-regulated molecules, *DAPL1* decreased by 3.8-fold, *SRCIN1* decreased by 3.3-fold, macrophage receptor with collagenous structure decreased by 2.4-fold, and fibroblast growth factor binding protein 1 (*FGFBP1*) decreased by 2.2-fold. The most involved pathways are shown in On-line Table 4. The most involved pathway was the atherosclerosis signaling pathway with 9 up-regulated genes compared with nontreated aneurysms. Similar to coiled aneurysms, agranulocyte adhesion and diapedesis pathways and the cell-to-cell signaling pathway were involved with flow diverters.

Table 2: Top up- and down-regulated molecules comparing flow-diverted versus untreated aneurysms, determined by IPA^a

Top Up-Regulated Molecules			Top Down-Regulated Molecules		
Gene Name	Description	Exp. Value	Gene Name	Description	Exp. Value
<i>PRND</i>	Prion protein 2 (dublet)	8.719	<i>DAPL1</i>	Death associated protein-like 1	−3.752
<i>KRT8</i>	Keratin 8	6.233	<i>SRCIN1</i>	SRC kinase signaling inhibitor 1	−3.295
<i>FGF23</i>	Fibroblast growth factor 23	5.662	<i>FAM150A</i>	Family with sequence similarity 150, member A	−2.555
<i>SIRPB1</i>	Signal-regulatory protein β 1	5.463	<i>MARCO</i>	Macrophage receptor with collagenous structure	−2.396
<i>CXCL8</i>	Chemokine (C-X-C motif) ligand 8	4.781	<i>LECT1</i>	Leukocyte cell derived chemotaxin 1	−2.373
<i>GPRI58</i>	G protein-coupled receptor 158	4.747	<i>SCIN</i>	Scinderin	−2.287
<i>MMP1</i>	Matrix metalloproteinase 1 (interstitial collagenase)	4.467	<i>FGFBP1</i>	Fibroblast growth factor binding protein 1	−2.183
<i>HMGA2</i>	High mobility group AT-hook 2	4.412	<i>MYH7B</i>	Myosin, heavy chain 7B, cardiac muscle, β	−2.107
<i>APLN</i>	Apelin	4.412			
<i>IL6</i>	Interleukin 6 (interferon, β 2)	4.375			

Note:—Exp. indicates expression.

^a Values are expressed as log-fold change.

Table 3: Top up- and down-regulated molecules comparing flow-diverted versus coiled aneurysms, determined by IPA^a

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

Note:—Exp. indicates expression.

^a Values are expressed as log-fold change.

Flow-Diverter-Treated versus Coiled Aneurysms

Using the criteria above for differential expression, we identified 13 of 9982 (0.13%) genes 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 IAs with coiled aneurysms, keratin 8 increased 4.3-fold, and basigin increased 3.8-fold. Protein disulfide isomerase-like was overexpressed in the coiled group compared with the flow-diverter group at 4.2-fold; and C-type lectin domain family 7, member A (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 performed in 5 selected genes from the top up- or down-regulated genes identified by RNA-seq. Those selected genes were *PRND*, fibroblast growth factor 23, *MMP1*, *SRCIN1*, *DAPL1*, and *HHIP*. Results obtained by reverse transcription polymerase chain reaction for gene expression levels varied in the same way and in comparable amplitude as those obtained with RNA-seq. Results of reverse transcription polymerase chain reaction are presented in On-line 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 biologic effects of coils and flow diverters and highlight pathways to better understand and optimize the outcomes after endovascular treatment of intracranial aneurysms.

Our results showed that relatively few genes were differentially expressed when comparing coiled versus flow-diverter-treated aneurysms. These findings show that despite 2 different approaches, the response to the device used for the endovascular treatment of IAs did not vary substantially. These findings further demonstrate that the observed gene modifications were mostly driven by the aneurysm itself rather than by the device and imply that the mechanisms leading to aneurysm occlusion are somewhat similar, regardless of the device used. However, some genes were differentially expressed in the flow-diverter-treated group compared with the coiled group.

Specifically, the most up-regulated molecule was keratin 8, which acts as a binding site for plasminogen.²⁹ This overexpression of plasminogen receptors could be deleterious to the treated aneurysms. Indeed, the 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 (*MMP-1*, 3, and 9).^{32,33}

Our study also found that apelin is up-regulated in flow-diverter-treated aneurysms compared with coiled aneurysms. This molecule significantly reduces aneurysm formation in the elastase model of abdominal aortic aneurysms 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 with coiled aneurysms; this overexpression 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 after the implantation of devices for the treatment of IAs. Metalloproteinases are known to be involved in thrombus homeostasis in IAs, 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) overexpressed in coiled versus untreated IAs. These *MMPs* have been reported as being implicated in abdominal aortic aneurysm formation and progression.⁴²⁻⁵² However their impact has not been extensively described in the setting of IAs.⁵⁰ We suspect that this increased level of *MMPs* in coiled aneurysms is linked to recanalization because *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) is up-regulated in flow-diverter-treated aneurysms compared with 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 *MMPs* in flow-diverter-treated aneurysms as previously described.¹⁵

Furthermore, the macrophage receptor with collagenous structure 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 walls of abdominal aortic aneurysms.⁵⁶ In our study, we observed a down-regulation of macrophage receptor with collagenous structure in the flow-diverter group, suggesting an increased implication of intra-aneurysmal thrombosis compared with 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 proinflammatory molecules⁵⁸ via multiple signaling pathways, including nuclear factor- κ B.⁵⁹ This involvement of the fibrinolytic system has been previously highlighted in abdominal aortic aneurysm pathology.⁶⁰ In abdominal aortic aneurysms, 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, which may play a

critical role in enlargement and rupture.⁵⁷ Furthermore, abdominal aortic aneurysm diameter is correlated with the level of plasmin activity in the abdominal aortic aneurysm wall.⁶¹ The overexpression of keratin 8 in flow-diverter-treated IAs could explain the deleterious issue in the rare cases of delayed aneurysm rupture after flow diverters. This overexpression of keratin 8 associated with a large amount of intrasaccular thrombus after flow-diverter placement supports intra-aneurysmal thrombosis as a possible cause of delayed aneurysm rupture after flow-diversion treatment³; however, the confirmation of this hypothesis would need further dedicated experiments to precisely determine the impact of keratin 8.

Another important function for aneurysm occlusion after endovascular treatment is wound healing, consisting subsequently of thrombus formation, myofibroblast invasion, and extracellular matrix deposition.^{5,12,62} C-type lectin domain family 7, member A (dectin 1) is a molecule promoting wound healing by the enhanced production of collagen matrices with β -glucans.⁶³⁻⁶⁵ Our results show that dectin 1 is approximately 4 times overexpressed in coiled IAs compared with flow-diverter-treated IAs. This finding suggests that wound healing is a process that is much more preponderant in coils than in flow-diverter treatment. *FGFBP1* is another molecule promoting wound healing.^{66,67} The present results show that *FGFBP1* is decreased in flow-diverter-treated aneurysms compared with untreated aneurysms; this decrease supports the idea that aneurysm occlusion after flow-diverter therapy is not related to wound-healing mechanisms but mostly to endothelial cell 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 IAs. However, this model has been evaluated with RNA-seq and has expression patterns similar to those in human intracranial aneurysms.¹⁶ However, this aneurysm model is not one of spontaneous rupture, and some biologic pathways may differ when considering rupture-prone aneurysms. To explore these mechanisms, analyzing gene expressions in new models for active aneurysms with inflamed aneurysm walls or bioactive thrombus would be interesting.^{70,71} Also, time intervals between the creation of an aneurysm and the time of euthanizing the animals were different between untreated and treated aneurysms, which could introduce a difference in the healing process. Given 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 reverse transcription polymerase chain reaction and will continue to explore these results further in other models.

Likewise, because of normal variations, there very likely are genes for which our threshold levels were not achieved, which may have an effect in humans. Just because a gene is not significantly up- or down-regulated 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 gene 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 that could be potential key factors in improving endovascular devices. This will require further validation with specific experiments to precisely describe the role of each molecule of interest.

CONCLUSIONS

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

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