Flow-dependent epigenetic DNA methylation in endothelial gene expression and atherosclerosis

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Abstract

Epigenetic mechanisms that regulate endothelial cell (EC) gene expression are now emerging. DNA methylation is the most stable epigenetic mark that confers persisting changes in gene expression. Not only is DNA methylation important in rendering cell identity by regulating cell type-specific gene expression throughout differentiation, but it is becoming clear that DNA methylation also plays a key role in maintaining EC homeostasis and in vascular disease development. Disturbed blood flow (d-flow) causes atherosclerosis while stable flow (s-flow) protects against it by differentially regulating gene expression in ECs. Recently, we and others have shown that flow-dependent gene expression and atherosclerosis development are regulated by mechanisms dependent on DNA methyltransferases (DNMT1 and 3A). D-flow upregulates DNMT expression both in vitro and in vivo which leads to genome-wide DNA methylation alterations and global gene expression changes in a DNMT-dependent manner. These studies revealed several mechanosensitive genes, such as HoxA5, Klf3, and Klf4, whose promoters were hypermethylated by d-flow, but rescued by DNMT inhibitors such as 5Aza-2-deoxycytidine. These findings provide new insight into the mechanism by which flow controls epigenomic DNA methylation patterns, which in turn alters endothelial gene expression, regulates vascular biology, and induces atherosclerosis.

Keywords

Epigenetics; DNA methylation; DNA Methyltransferases; gene expression; flow; shear stress; endothelial cells; atherosclerosis

Introduction

The discovery of disease master regulators that control a large network of genes is of high interest to vascular biologists because such biomolecules would serve as excellent therapeutic targets for disease treatment and prevention. Potentially important master regulators include epigenetic modifiers and transcription factors that participate in pre-
transcriptional regulation, microRNAs involved in post-transcriptional regulation, and post-translational modifiers. Epigenetic modifications such as DNA methylation, histone modifications, and chromatin remodeling complexes, alter the genomic DNA structure and accessibility. Putative master regulators of vascular biology, including chromatin structure remodelers, epigenetic DNA modifications (cytosine modifications including DNA methylation, and histone modifications), and microRNAs have recently been the subject of extensive research to determine their role in large-scale gene network regulation in endothelial cell biology and cardiovascular disease.

1. The role of flow in atherosclerosis and endothelial cell function

Blood flow generates shear stress on the vascular walls. Unidirectional, laminar shear stress (LS), or stable flow (s-flow) is crucial for normal vascular function, whereas disturbed flow (d-flow), characterized by low and reversing oscillatory shear stress (OS), causes endothelial dysfunction and atherosclerosis. ECs have dramatically altered gene expression patterns when exposed to d-flow versus s-flow. Atherosclerosis preferentially develops in areas of d-flow, where the dysfunctional EC phenotype initiates and perpetuates plaque accumulation.

2. Flow-mediated gene expression

Shear stress regulates endothelial gene expression in vitro and in vivo. ECs have numerous mechanoreceptors that sense changes in shear stress direction and magnitude which initiates a complex cascade of signaling events that alters global gene expression and regulates endothelial structure and function. The net effect of s-flow is to upregulate “atheroprotective” genes and downregulate “pro-atherogenic” genes, while the net effect of d-flow is the opposite: upregulation of pro-atherogenic genes and suppression atheroprotective genes. However, the mechanism by which d-flow causes changes in EC gene expression is still incomplete.

3. Epigenetics introduction

Epigenetics is defined as the modification of genetic information in a sequence-independent manner and it may be mediated by altering DNA conformation. Genomic DNA in an open, relaxed conformation (euchromatin) is associated with acetylated histones, unmethylated DNA, and active transcription, whereas condensed heterochromatin is associated with repressive marks such as trimethylated-histone 3 lysine 9 (H3K9), trimethylated-histone 3 lysine 27 (H3K27), and DNA methylation. Heterochromatin generally contains repeat elements, imprinted, and transcriptionally silent genes, and its compaction predominantly occurs during development and differentiation.

Shear stress has been found to mediate chromatin remodeling and histone modifications and this is thought to play a role in shear-induced gene expression changes. LS activates histone acetyltransferase (HAT) activity, deactivates histone deacetylases (HDACs), and induces histone H3/H4 acetylation and H3 phosphorylation in cultured ECs, and this regulates the key EC transcription factors Kruppel-like Factors 2 and 4 (Klf2, Klf4), and endothelial nitric oxide synthase (eNOS), via myocyte enhancer factor-2 (MEF2).
Conversely, OS causes HDAC overexpression, and HDAC inhibition prevents OS-induced EC proliferation and EC inflammation in vivo and in vitro.

DNA methylation involves the addition of a methyl group to the 5′ carbon of a cytosine base pair (5-methylcytosine, 5mC), which occurs most frequently in the context of a CG dinucleotide. CpG islands are dense regions of CG sites with higher than expected CG content (generally defined as > 50% with an observed vs. expected ratio of >0.6, over a distance of at least 200 base pairs). Approximately 40% of human genes are associated with CpG islands, which are normally unmethylated, but transcriptionally repressed regions are generally highly methylated. CpG sites are otherwise sparse throughout the genome due to a high rate of C to T mutation caused by spontaneous deamination of methylated cytosine, and only functionally important CG sites are likely to be evolutionarily protected from this mutation.

DNA Methyltransferases (DNMTs) catalyze the addition of the methyl group to cytosine. DNMT1 is thought to preferentially methylate hemi-methylated DNA. Thus, the complementary strand of a CG dinucleotide, which is a mirroring GC dinucleotide, usually carries the same methylation markings as its CG partner. Although DNMT1 is classically referred to as a maintenance methylase, it also has de novo methylation capabilities. Both DNMT1 overexpression and DNMT1 deficiency are embryonic lethal, and DNMT1 deletion causes genome-wide hypomethylation while DNMT1 overexpression causes genome-wide hypermethylation. DNMT3a and DNMT3b are classically referred to as de novo methyltransferases that preferentially add methyl groups to fully unmethylated DNA. DNMT3A and DNMT3B establish global DNA methylation patterns during embryogenesis and gametogenesis.

CG methylation in a gene promoter, close to the transcription start site, generally suppresses gene expression. Promoter methylation is thought to inhibit gene transcription in two ways: first, by physically impeding transcription factor binding to the gene promoter, and second, by binding to methyl-CpG-binding domain proteins which recruit repressive machinery such as histone and chromatin modifiers to the locus that cause chromatin compaction. The detailed crosstalk between DNA methylation and histone modifications remains a topic of extensive research. However, the role of DNA methylation in the gene body is less straightforward.

4. DNA Methylation in endothelial cell development and disease

Global DNA methylation patterns change dramatically over the course of cell differentiation and the stable epigenetic marks of terminally differentiated cells govern their gene expression and function. EC biology differs across vascular beds based on the functional requirements in a given anatomical region. Differences in EC subtype gene expression are regulated, in part, by epigenetic DNA methylation and histone modifications. Similar differences may also be extended to endothelial dysfunction and atherosclerosis.

Aberrant epigenetic patterns of DNA methylation and histone modifications are hallmarks of diseases such as cancer. Although atherosclerosis shares many phenotypic characteristics...
with cancer, including hyperproliferation, migration, and inflammation, whether those abnormal epigenetic changes also occur in atherosclerosis is unclear.

Drugs that inhibit DNA methyltransferases have proven to be promising treatment options for many cancers. 5Aza-2′-deoxycytidine (5Aza) is a well-studied DNMT1 preferential inhibitor. 5Aza is a nucleoside analog that works by trapping DNMT1 in a covalent complex with DNA, resulting in DNMT1 degradation. 5Aza, also known as Decitabine, is used as a chemotherapeutic agent for acute myeloid leukemia and myelodysplastic syndromes. However, its cytotoxicity limits its therapeutic capacity.

5. Flow-dependent DNA methylation in endothelial biology and atherosclerosis

Recently, three groups working independently and using different model systems converged on a seminal finding that DNA methyltransferases are regulated by shear stress and that they regulate flow-mediated endothelial gene expression and function as well as atherosclerosis.

Jiang et al. showed that flow regulates endothelial Klf4 transcription by DNMT3A-mediated DNA methylation using porcine aorta and cultured human aortic endothelial cells. Klf4 is a key mediator of endothelial function and has been well documented to maintain an anti-inflammatory, quiescent EC state in unidirectional flow conditions. They showed that d-flow increases expression of DNMT3A, which in turn induces Klf4 promoter hypermethylation thereby decreasing MEF2 binding to the promoter and ultimately suppressing Klf4 transcription, in a manner reversible by the DNMT inhibitors 5Aza and RG108. Moreover, RG108 treatment reversed the d-flow-induced loss of downstream Klf4 targets eNOS and thrombomodulin (THBD), and the d-flow-induced overexpression of the monocyte chemoattractant protein-1 (MCP-1). They validated that d-flow induces MEF2 binding site hypermethylation in the Klf4 promoter in vivo using endothelium isolated from swine aorta. This work uncovered a novel epigenetic regulatory mechanism for the key EC gene Klf4.

Our group aimed to discover whether d-flow could cause DNA methylation changes that would alter gene expression globally and promote atherosclerosis. Dunn et al. performed genome-wide studies of DNA methylation and gene expression using reduced representation bisulfite sequencing (RRBS) and microarray, respectively. We found that d-flow regulates genome-wide DNA methylation patterns in a DNMT-dependent manner. Using partial carotid ligation surgery in a murine model to induce d-flow, we found that DNMT1 expression is upregulated by d-flow in ECs both in vivo and in vitro. Further, 5Aza or DNMT1 siRNA markedly reduced OS-induced endothelial inflammation in human umbilical endothelial cells (HUVECs). Moreover, 5Aza treatment drastically prevented atherosclerosis development in two different models of murine atherosclerosis – the partial carotid ligation and the standard high-fat diet model. More recently, Cao et al. have shown a similar anti-atherogenic effect of 5-Aza using LDLR−/− mice.

Zhou et al. also reported that d-flow causes DNMT1 overexpression. Using HUVECs, they found that OS increases DNMT1 expression, DNMT1 nuclear translocation, and 5mC content in a 5Aza-inhibitable manner. They also used a rat partial carotid ligation model and showed that d-flow induced DNMT1 protein expression and increased 5mC content.
We further carried out additional studies to determine genome-wide DNA methylome changes and gene expression using the partial carotid ligation model with or without the 5Aza treatment. These two DNA methylome and transcriptome datasets were analyzed by systems biological approaches. Our initial analysis focused on finding genes that are hypermethylated in their promoter regions and have downregulated gene expression by d-flow in a 5Aza-reversible manner. This analysis revealed 11 genes, *HoxA5*, *Klf3*, *Tmem184b*, *Adams15*, *Cmklr1*, *Pkp4*, *Acvrl1*, *Dok4*, *Spry2*, *Zfp46*, and *F2rl1* (Table 1). Interestingly, 5 of them (*HoxA5*, *Klf3*, *Cmklr1*, *Acvrl1*, and *Spry2*) contain C-AMP response elements (CRE) in their promoters. *HoxA5* and *Klf3* encode transcription factors and thus the methylation status of these loci could serve as a mechanosensitive master switch in gene expression. The biological function of those genes is currently under investigation.

Together, these results demonstrated that d-flow controls epigenomic DNA methylation patterns in a DNMT-dependent manner, which in turn alters endothelial gene expression and induces atherosclerosis. This study generated new knowledge about the epigenetic EC flow response and uncovered novel therapeutic gene targets for further study.

Genome-wide DNA methylation studies can be used as a discovery platform to reveal novel flow-sensitive genes. Our study was the first to report that *HoxA5* and *Klf3* are mechanosensitive transcription factors. Homeobox (Hox) genes are highly conserved, known to be important in development and dysregulated in cancer, and are controlled by DNA methylation. Hox family members have been implicated in vascular remodeling, angiogenesis, and disease by orchestrating changes in gene expression, the extracellular matrix, and integrins. *HoxA5* is known to regulate various endothelial functions such as migration, angiogenesis, and inflammation. *Klf3*, unlike the other two well-known known mechanosensitive transcriptional activators *Klf2* and *Klf4*, is known to repress transcription. Interestingly, *Klf3* is suppressed in acute myeloid leukemia (AML), and 5Aza is used to treat this disease. AML is also treated with all-trans retinoic acid, which was shown to rescue *Klf3* expression by an unknown mechanism. It would be interesting to determine whether the therapeutic effects of 5Aza and all-trans retinoic acid on AML are mediated, at least in part, by rescuing *Klf3* promoter hypermethylation.

Atherosclerosis develops due to dysfunction of multiple cell types, including ECs, smooth muscle cells (SMCs), immune cells, and fibroblasts, and the anti-atherogenic effects of 5Aza have been shown to be mediated by not only ECs, but also by immune cells.

### 6. Relationship between histone modification and DNA methylation and epigenetic crosstalk

Flow mediated gene expression is regulated by both genetic (sequence-dependent transcription factor binding) as well as epigenetic (histone modifications and DNA methylation) mechanisms (Figure 1). Although it has not been directly demonstrated under different flow conditions in endothelial cells, it is well established that epigenetic mechanisms involving histone modifications (e.g., acetylation, deacetylation, methylation, phosphorylation) and DNA methylation interact and influence one another, affecting overall gene expression activity. It is also likely that interaction of these epigenetic and genetic mechanisms leads to an orchestrated net effect of s-flow (or LS) and d-flow (or OS) on
mechanosensitive gene expression in ECs. The importance and extent of these interactions remains to be determined. Our working model is that DNA methylation in the promoter regions by d-flow leads to chronic repression of anti-atherogenic genes (epigenetic mechanisms), while pro-atherogenic gene expression is mediated by regulation of transcription factors (genetic pathways). In contrast, s-flow rescues (de-represses) expression of anti-atherogenic genes by decreasing DNA methylation in the promoter regions via reduced DNMT activity and altered histone modifications. S-flow also regulates genetic pathways by regulating transcription factor binding to specific gene promoter sites, leading to increased anti-atherogenic genes and decreased pro-atherogenic genes.

Most of the DNA CpG sites are methylated during development by DNMT3a and DNMT3b with CpG islands being subject to differential methylation affected by various conditions. There have been numerous reports describing the interaction between histone modifications such as acetylation and methylation (ex: by G9a, or SET domain containing histone methyltransferases) with DNA methylation and repression of transcription. DNMT3A and DNMT3B share an unmodified-histone binding site. H3K4 methylation is associated with decreased DNA methylation in multiple cell types, whereas H3K9 methylation and histone deacetylation are associated with increased DNA methylation which leads to gene repression by forming heterochromatin. DNMT3A and DNMT3B also harbor domains that read H3K36me3 which is found in the bodies of certain genes and it is thus speculated that they play a role in gene body methylation which is abundant but not very well understood. Moreover, DNMT1 also recognizes H3K23 monoubiquitination which seems to play a role in maintenance of DNA methylation.

Emerging evidence indicates that DNA methylation can also affect histone modifications via interactions of DNMTs with histone methyltransferases and deacetylases. For example, CpG methylation enhances UHRF1 (Ubiquitin-like containing PHD and RING finger domains 1, a DNMT1 recruiter) and perturbs KDM2A (Lysine-specific demethylase 2A, a H3K36 demethylase enzyme) binding to H3K9me3-containing nucleosomes. Also, DNA methylation causes H3K9 demethylation, which is a marker of repressive chromatin, by interaction of G9a and DNMT1. DNA methylation also inhibits H3K4 methylation which is a marker of active transcription. This is supported by the fact that unmethylated DNA is mainly assembled in nucleosomes that contain acetylated histones, while methylated DNA is assembled in nucleosomes containing acetylated H3 and H4 which leads to more compact chromatin. Moreover, there have been reports that methylcytosine-binding proteins, such as meCP2 or mbD2 might help recruit HDACs and thus lead to histone acetylation.

It is becoming clear that an interplay exists among methylation, miRNAs and histone acetylation. Hypermethylation of multiple miRNA promoter sites (ex: miR-1, 26a and 137) has been reported in multiple cancer types. Moreover, HDACs (HDAC1-9 and 11) are direct targets of miRNAs, while HDACs have shown to regulate expression of miRNAs (e.g., miR-15-a/16-1, 183, 224, 449a). In ECs, Klf4 and Klf2 are two key flow-sensitive genes that are co-regulated by miR-92, and regulate expression of multiple microRNAs as well. As we described above, Klf4 is also controlled by flow-dependent DNA methylation. This opens up the interesting possibility of cooperation between flow-mediated epigenetic events.
Perspectives

Emerging studies clearly demonstrate that d-flow regulates epigenomic DNA methylation by upregulated DNMTs in ECs, which in turn alters endothelial gene expression and function, ultimately contributing to atherosclerosis. The advent of new technologies to resolve genome-level methylation at the nucleotide scale combined with advanced systems biology approaches will continue to play a key role better understanding the effects of DNA methylation in gene network regulation, endothelial biology, and vascular disease. Currently, DNA methylation studies mainly focus on studying 5mC, but other modifications such as 5-hydroxymethylcytosine (5hC), 5-formylcytosine (5fC) and 5-carboxycytosine (5cC) are also likely to be important. In addition, demethylating enzymes such as Ten-eleven-translocation (TET) may also play an important role in regulating gene expression in this disease.

Experimental methods to interrogate the epigenome of flow-regulated ECs involve the use of either in vivo-derived or in vitro cultured cells. In vivo methods have a clear benefit due to biologically artificial conditions of cell culture, which are known to have epigenetic consequences. For example, ECs in vivo are constantly exposed to flow, whereas cultured ECs must be exposed to static, no-flow conditions during the culture process and non-adherent conditions during passaging. These intermediate mechanical conditions can have dramatic effects on long-term epigenetic state. Gene expression studies by microarray using cultured ECs demonstrate that in vitro biological responses are often quite different from events that occur in vivo98. This raises an important consideration, especially persisting epigenetic changes, for the limitations of in vitro studies and points to the major benefit of models that utilize in vivo-derived genomic DNA with no intermediate cell culture steps.

There are several methods available to interrogate genome-wide DNA methylation patterns. The most comprehensive is whole genome bisulfite sequencing, but its high cost and the large amount of data generated, including poorly understood intergenic “desert” regions, currently makes whole genome sequencing a less desirable method. Alternative methods with higher efficiency and practicality to resolve the endothelial DNA methylome include methylation microarrays and reduced representation bisulfite sequencing (RRBS) methods. Methylation microarrays are limited by the probe set and only examine regions defined by previous knowledge (such as promoter- and CpG island-confined CpG sites). RRBS is currently a gold standard for genome-scale methylation studies at the nucleotide resolution, although incomplete CG coverage is an inherent limitation of RRBS due to the MspI restriction enzyme digest, PCR, and sequencing steps99. Both methylation microarrays and RRBS produce partial genomic coverage, which may result in overlooking potentially important methylation sites. Compared to currently existing technologies, RRBS is a much more comprehensive but cost-effective method to interrogate genome-wide DNA methylation.

While most work in this realm has been mainly focused on CpG methylation in the promoter region of genes, DNA methylation in other locales, including coding or noncoding gene regulatory regions, and at other motifs such as CpH (where H is A/T/C), are known to be important for controlling gene expression, alternative transcripts, chromatin conformation
and genome stabilization \textsuperscript{61, 62, 100, 101}. Additionally, while DNA methylation at the 5′ carbon of a cytosine base pair is the most well studied epigenetic covalent DNA modification, several others are known to exist and were recently implicated to be important in gene expression regulation, although their mechanisms are very unclear. These modifications include 5-hydroxymethylcytosine (5hC), 5-formylcytosine (5fC) and 5-carboxycytosine (5cC)\textsuperscript{102}. Emerging methods of detection will enable future study of these cytosine modifications in endothelial biology and pathogenesis \textsuperscript{103}.

Much remains to be determined about the nature of DNA methylation changes in response to flow; How does d-flow regulate DNMT expression and what controls flow-dependent DNA demethylation? What are the functional consequences of DNA methylation at the genome-wide and individual gene levels? How does DNA methylation at a specific promoter site affect transcription of the flow-sensitive genes? It is also important to compare and translate mouse data to clinical data, with important limitations to experimental models, especially since human atherosclerosis develops over decades and is multifactorial in nature. For example, the study of epigenetic factors involved in atherosclerosis would expectedly become more complicated in humans due to differences in life habits such as smoking, sedentary life styles as well as other comorbidities such as hypertension, diabetes and obesity. There have been conflicting reports showing global hypomethylation in human atherosclerotic plaques \textsuperscript{104}, while other studies in contrast showed hypermethylation across many genomic loci in diseased aortas \textsuperscript{105}, indicating that more studies are needed. Given the known side effects of using DNMT inhibitors such as 5Aza as therapeutics, future studies are needed to identify additional downstream target genes that may be more effective therapeutic points of intervention with reduced undesirable effects.

**Significance**

These emerging findings surrounding the endothelial cell epigenetic response to flow provide new insight into the mechanism by which flow controls epigenomic DNA methylation, which in turn regulates endothelial gene expression, vascular biology, and ultimately atherogenesis. Based on our recent mouse DNA methylome study, it is tempting to speculate that physical inactivity increases atherosclerosis risk partly because it is associated with d-flow, which leads to chronic repression of anti-atherogenic genes by the epigenetic DNA methylation-dependent mechanism. In contrast, the well-known anti-atherogenic effect of aerobic exercise may be due in part mediated by de-repression of those anti-atherogenic genes caused by DNA methylation. The discovery of endothelial cell disease master regulators that control a large network of genes, such as DNA Methyltransferases, and their specific target genes is important to enable further development of therapeutics for vascular disease treatment and prevention.

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References


44. Li M, Chen SS. The tendency to recreate ancestral cg dinucleotides in the human genome. BMC evolutionary biology. 2011; 11:3. [PubMed: 21208429]


Figure 1. Flow regulates both genetic and epigenetic pathways involved in gene expression control – Working model

(A) Stable flow (s-flow) downregulates DNA Methyltransferases (DNMTs) and histone deactylases (HDACs), and upregulates histone acetyltransferases (HATs). MicroRNAs (miRNAs) and transcription factors (both transcriptional activators (TAs) and transcriptional repressors (TRs) are also regulated by flow and demonstrate molecule-specific, coordinated responses. The interplay of these events leads to orchestrated transcription of a subset of anti-atherogenic genes and also prevents the expression of pro-atherogenic genes. (B) Under d-flow conditions, the opposite responses occur: HATs are downregulated and HDACs and DNMTs are overexpressed, leading to repression of a subset of anti-atherogenic genes and increased expression of a subset of pro-atherogenic genes. This mechanosensitive reprogramming of gene expression by epigenomic DNA methylation leads to atherosclerosis development.
Figure 2.
(A) Stable flow (s-flow) downregulates expression of DNA Methyltransferases (DNMTs) which allows the promoter of anti-atherogenic genes such as *Klf4* and *HoxA5* to remain demethylated, enabling their expression. (B) Disturbed flow (d-flow) upregulates DNMT expression, leading to hypermethylation of the promoter of anti-atherogenic genes such as *Klf4* and *HoxA5*, repressing their expression.
The subset of flow-sensitive genes described here have dramatic d-flow induced promoter hypermethylation corresponding to suppressed gene expression, both of which can be reversed by 5Aza treatment.

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Type</th>
<th>Known Function</th>
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<tbody>
<tr>
<td>Acvrl1</td>
<td>serine/threonine kinase receptor</td>
<td>Type I cell-surface TGFβ receptor that may be involved in hemorrhagic telangiectasia, pulmonary arteriovenous malformations, and severe pulmonary arterial hypertension</td>
</tr>
<tr>
<td>Adamtsl5</td>
<td>ECM-binding protein</td>
<td>Involved in fibrillin and heparin binding and metalloendopeptidase activity</td>
</tr>
<tr>
<td>Cmkrl1</td>
<td>G-protein-coupled receptor</td>
<td>Leukotriene B4 (inflammatory cytokine) receptor that can activate the transcription factor PPARα. May play a role in cardiac muscle contraction and lipid metabolism, and is implicated in pancreatic cancer.</td>
</tr>
<tr>
<td>Dok4</td>
<td>Scaffolding protein</td>
<td>Docking platform for assembly of multimolecular signaling complexes. May be involved in T-cell immune regulation, phospholipid binding, and insulin receptor binding.</td>
</tr>
<tr>
<td>F2rl1</td>
<td>G-protein-coupled receptor</td>
<td>Proteolytically-activated receptor that modulates inflammatory responses.</td>
</tr>
<tr>
<td>HoxA5</td>
<td>Transcription Factor</td>
<td>Differentiation, development, cancer, vascular biology</td>
</tr>
<tr>
<td>Klf3</td>
<td>Transcription Factor</td>
<td>Predominantly acts transcriptional repressor. Involved in erythroid cell maturation and adipogenesis</td>
</tr>
<tr>
<td>Pkp4</td>
<td>Junctional protein</td>
<td>Armadillo-like protein that plays a role as a regulator of Rho activity during cytokinesis. May play a role in desmosomal junctional plaque organization and cadherin function.</td>
</tr>
<tr>
<td>Spry2</td>
<td>Receptor tyrosine kinase signaling protein</td>
<td>Bimodal regulator of epidermal growth factor receptor/MAPk signaling. May function as an antagonist of fibroblast growth factor pathways and may negatively modulate respiratory organogenesis.</td>
</tr>
<tr>
<td>Tmem184b</td>
<td>Transmembrane Protein</td>
<td>Mostly unknown, but may activate mitogen-activated protein kinase (MAPk) signaling</td>
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<td>Transcription Factor</td>
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