

Brief Report

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Brief Report

Microarray Analysis Shows Important ID3–BMP9 Driven Gene Signatures

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Abstract: Bone morphogenetic protein 9 (BMP9) is a cytokine belonging to the transforming growth – beta (TGF- β) family mainly known for its role in regulating vascular function, specifically keeping blood vessels stable and preventing unwarranted growth. It has been previously demonstrated to be involved in hereditary hemorrhagic telangiectasia (HHT) and vascular remodeling acting via HHT target genes. Inhibitor of Differentiation/DNA – Binding 3 (ID3) has been known to be a significant mediator for stages of cell differentiation within the context of BMP9 signaling in HHT development; however, the roles of ID3 and its targets within the HHT pathways are still limited. This brief report highlights significant ID3 targeted - BMP9 gene signatures and networks that may play a key role in the modifications of HHT.

Keywords: BMP9; gene signatures; ID3; microvascular

Introduction

Bone morphogenetic protein 9 (BMP9) is a member of the transforming growth – beta (TGF- β) superfamily and regulates various cellular processes, including: osteogenic differentiation, angiogenesis, and metabolism. Among the BMP family members, BMP9 is one of the most potent inducers of osteogenesis. BMP9 signals mainly through the ALK1 receptor and activates the SMAD1/5/8 pathway, leading to the transcriptional regulation of genes involved in proliferation and differentiation [1–3]. Inhibitor of Differentiation/DNA-binding 3 (ID3) is part of the ID protein family, which acts as dominant-negative regulators of basic helix-loop-helix (bHLH) transcription factors. Lacking a DNA-binding domain, ID3 functions by forming heterodimers with bHLH factors, thereby inhibiting their interaction with DNA. ID3 is associated in the regulation of differentiation, cell proliferation, and vascular development; and is transcriptionally regulated by several pathways, including BMP signaling [3–8]. The interaction between BMP9 and ID3 represents a key regulatory axis in controlling cell fate. BMP9 has been shown to upregulate ID3 expression via SMAD-dependent mechanisms, suggesting that ID3 may act as a downstream effector of BMP9 signaling. This regulatory relationship contributes to the modulation of differentiation processes, particularly in osteogenic and endothelial contexts [4–11]. However, data establishing ID3/ID3 targets and BMP9 connections within HHT context is limited. This study shows the interactions between ID3, ID3 curated target genes, and differential expressed genes (DEGs) significant in the endothelial environment. The results shown in this study will help open up additional possibilities toward diagnostic targets for HHT within the focus of BMP9 and ID3 pathways.

Methods

ID3 and ID3 target genes were curated and used from results highlighted in previous research [12]. To create significant links between ID3, ID3 targets, and differentially expressed genes (DEGs) within a BMP9 treated environment; microarray expression data from NCBI Gene Expression Omnibus (GEO) database were used (accession number: GSE40960) [13]. The original study information consisted of 6 samples (3 BMP9 treated and 3 untreated human dermal microvascular endothelial cells). Using the Limma-Voom R package, we identified DEGs and showed the expression

density (Figure 1) as seen below [14]. We further examined the gene interactions between ID3, the ID3 target genes, and the top DEGs through GeneMania, a software that helps predict the interaction and function of genes [15].

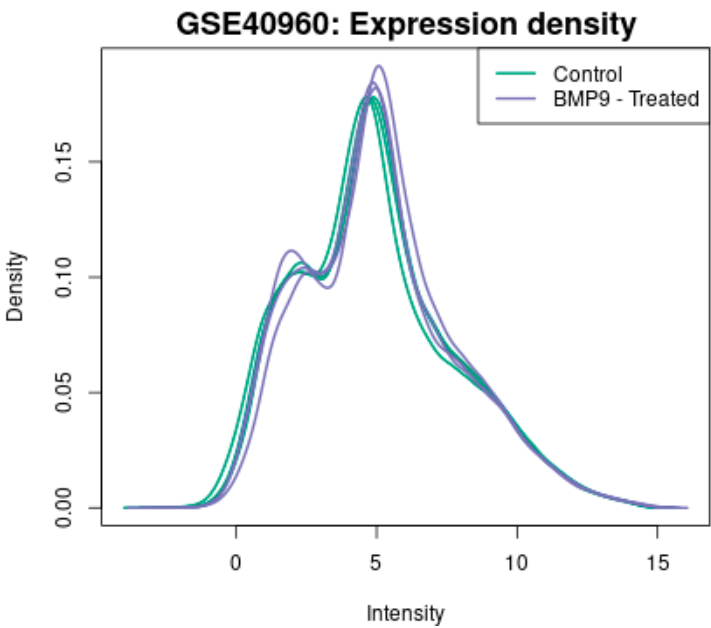


Figure 1. The expression density plot displays the distribution of gene expression values in the microarray data to assess if the expression distributions are normalization and cross – comparable. The graph shows the plot of control (3) and BMP9 – treated (3) samples.

Results

ID3 and ID3 target genes were expressed in the data that included 11 genes: ID3, MCM4, NDUFA7, ABCB6, DNMT1, DHRS3, DCBLD2, CDH15, CAD, BYSL, and ACP1 [12]. Microarray data from GSE40960 was furthermore used to investigate significant gene signatures. We performed an analysis of DEGs, and results showed DEGs with significant fold changes (Table 1). These genes included: CCDC144B, NXPH1, PCDH11X, LOC285835, TAAR1, ZPBP2, ID1, LILRB5, HGFAC, TMEM161B, LIAS, IL32, C10orf44, UNC93A, PDGFRA, ARHGDIA, PRR16, LOC100132891, FLJ30838, and IZUMO1. Overall, these genes were recorded into GeneMania and showed a fully connected structure that displayed various gene networks with co – expressed and predicted networks having the strongest connections (Figure 2).

Table 1. Top 20 DEGs based on fold change (up – and down – regulated genes).

Gene	Fold Change
CCDC144B	23.89865699
NXPH1	16.22331458
PCDH11X	16.08455194
LOC285835	15.70455983
TAAR1	14.77448089
ZPBP2	14.44781726
ID1	13.76492843
LILRB5	12.90671705
HGFAC	12.26808441
TMEM161B	12.14174311

LIAS	0.081963901
IL32	0.091010293
C10orf44	0.092433913
UNC93A	0.099472054
PDGFRA	0.100139557
ARHGDIA	0.103217557
PRR16	0.105769262
LOC100132891	0.109581022
FLJ30838	0.11039034
IZUMO1	0.110476869

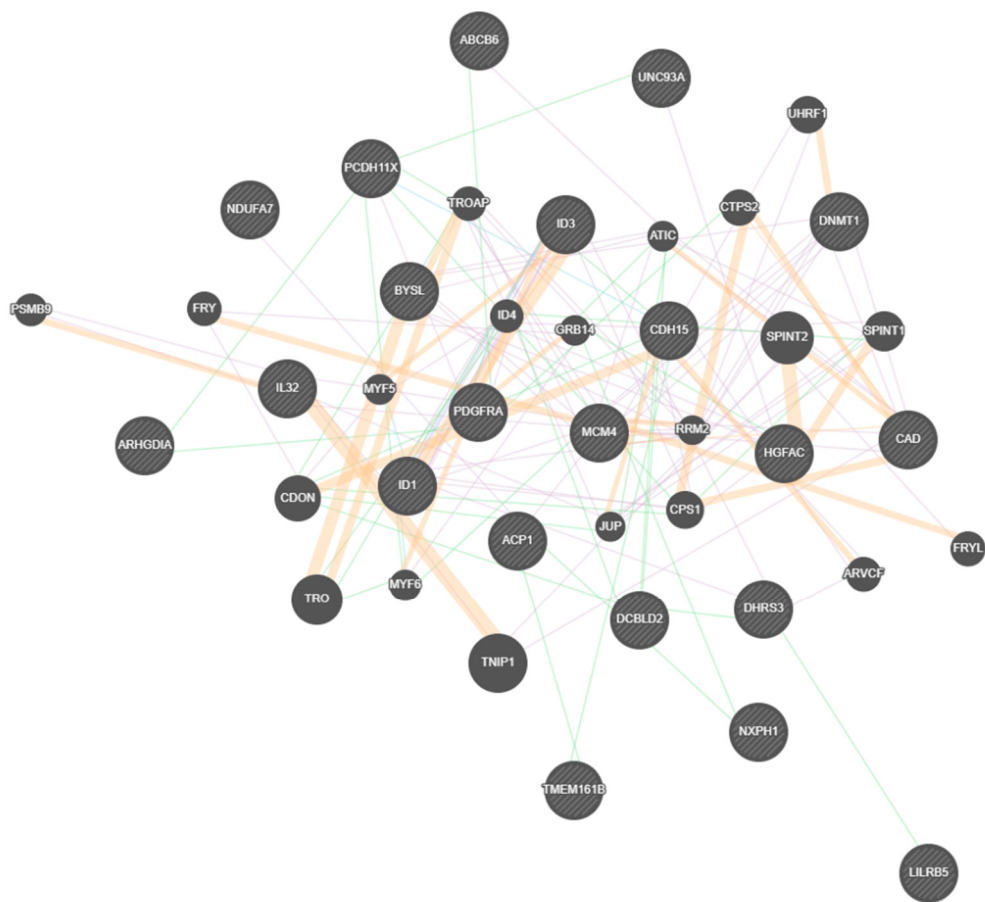


Figure 2. Fully connected and interacting gene network of ID3, ID3 target genes, and the top 20 DEGs (up – and down – regulated genes based on fold change) using GeneMania. The gene network shows co – expression (66.27% in purple), predicted (28.53% in orange), co – localization/pathway (3.15% in blue), and genetic interactions (2.05% in green).

Discussion

Overall, the results from this study add to the evidence that ID3, ID3 target genes, and key DEGs are important for potential targets toward HHT. By using a variety of tools including the R – package, NCBI GEO, GeneMania, and results from previous research, we discovered important ID3 – BMP9 driven genes and gene – gene networks [12]·[14]·[15]. Previously, it was shown that ID3 and ID3 targets such as MCM4, NDUFA7, ABCB6, DNMT1, DHRS3, DCBLD2, CDH15, CAD, BYSL, and

ACP1 were included in the gene pathways and signaling of different vascular malformations such as HHT [7,12,16]. However, with the new data that shows interactions with these established targets and novel DEGs, this helps to build a stronger foundation for additional investigations within ID3 and BMP9 pathways toward HHT. One limitation for this study is the overall small sample size that is shown in the data gathered from NCBI GEO which may restrict the statistical power. In order to address this, larger sample sizes are needed for future studies to avoid the risk of type 2 errors and bias.

Conclusion

Our results show the links and interactions between ID3, ID3 targets, and significant DEGs in the microarray data. Through the combined genomic and bioinformatics analysis, we represented DEGs and gene - to - gene networks that improve the understanding with ID3 and BMP9 signaling. Additional research needs to be focused on known transcriptional regulators such as ID3 and how their impact with BMP9 contributes to modifications within HHT. These innovations can better inform clinicians, researchers, and public health professionals about HHT and tools to combat it for the future.

Author Contributions: V. A. conceptualized, designed, conducted, and wrote the manuscript.

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Data Availability Statement: The data used for the analysis was deposited in NCBI GEO (GSE40960). Any additional questions about this study can be directed toward the corresponding author.

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

Statement of Ethics: An ethics statement was not required because this study is based on publicly deposited and accessible data.

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