INHIBITION OF APELIN EXPRESSION BY BMP SIGNALING IN ENDOTHELIAL CELLS

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Abstract

The TGF-beta/bone morphogenic protein (BMP) system, is a major pathway for angiogenesis and is involved in hereditary vascular diseases. Here we report that the gene encoding the vaso-active and vascular cell growth-regulating peptide apelin is a target of the BMP pathway. We demonstrate that apelin expression is strongly down-regulated by BMP in an endothelial cell line as well as in lung endothelial microvascular cells. We show that BMP signals through the BMPR2-SMAD pathway to down-regulate apelin expression and that a transcriptional direct and indirect mechanism is required. The BMP-induced down regulation of apelin expression was found to be critical for hypoxia-induced growth of endothelial cells, because the growth inhibitory effect of BMP in this condition is suppressed by enforced expression of apelin. Thus, we describe an important link between a signaling pathway involved in angiogenesis and vascular diseases and a peptide regulating vascular homeostasis.

Key-words:

Pulmonary endothelial cells, hypoxia, SMAD.
Bone morphogenetic proteins (BMPs) are a subgroup of the transforming growth factor-β (TGF-β) super-family of growth factors (37) and signal through heteromeric complexes of type I (ALK1, 2, 3) and type II (BMPR-II, ACVR2A or ACVR2B) serine/threonine kinase receptors. After the BMPs have bound to the receptor complex, the type II receptors activate the type I receptors by phosphorylation, which then activate intracellular signaling pathways. Upon BMP stimulation, SMAD1, 5 or 8 are phosphorylated by the BMP type I receptors, then bind to a common SMAD partner, SMAD4. The complex translocates to the nucleus and modulates target gene transcription (33). Two inhibitory SMADs, SMAD6 and SMAD7 are themselves induced by BMPs and inhibit BMP signalling (19, 39), thus providing a negative feedback loop. Alternative signalling pathways through p38MAPK, JNK, and ERK activation have been documented (49), as well as activation of the miRNA, miR-21, leading to the decrease of RNA targets such as dedicator of cytokinesis – DOCK 180 related proteins (21).

Few transcriptional target genes of BMPs have been identified and include Id1, Id2, TBX20 (32), and VEGFA (44).

BMPs have been shown to play an important role in vascular development and homeostasis (28, 35). Heterozygous ACVRL1 or ENG mutations cause hereditary hemorrhagic telangiectasia, a severe angiodysplasia affecting capillary and large vessel formation. Mono-allelic BMPR2 and ACVRL1 mutations are responsible for the heritable form of pulmonary hypertension, a rare and severe disorder characterized by elevated pulmonary vascular resistance due to vasoconstriction and medial hypertrophy of small pulmonary arteries (36).

BMPR1A (ALK3) and SMAD4 heterozygous mutations cause juvenile polyposis of the colon, associated with HHT in the case of Smad4 mutations. BMP pathway genes disruptions in mice have demonstrated their major roles in embryonic angiogenesis. Indeed, ACVRL1 or
ENG inactivations in mice induce major vasculogenesis impairment and embryos die at midgestation (27, 34), whereas mice with a homozygous deletion of BMPR2 die earlier and lack mesoderm (3).

Using a genomic approach for identifying BMP target genes, we found that the vaso-active and angiogenic peptide apelin is strongly down-regulated by BMP signaling in endothelial cells at the transcriptional level, and through the smad pathway. Apelin, the mature peptide cleaved from preproapelin is active mainly as a 13 aminoacid active peptide, and signals through the apelin receptor (APLNR), which is present in the heart and pulmonary vessels, and activates NO-dependent vasodilatation and increases cardiac contractility. Apelin gene (APLN) expression is strongly induced by hypoxia (13). The BMP-apelin regulatory axis was found to be critical for hypoxia-induced growth of endothelial cells, because the growth inhibitory effect of BMP9 in this condition is suppressed by enforced expression of apelin. These results identify an important regulatory link between BMP and the apelin-APLNR vascular signaling pathway.
Materials and methods

Reagents. Recombinant human BMP4, BMP7 and BMP9 were purchased from (R&D Systems, Minneapolis, MN). Cycloheximide and actinomycin D were obtained from (Boehringer Ingelheim GmbH, Ingelheim, Germany).

Cell culture. The human dermal microvascular endothelial cell line HMEC-1 was obtained from Thomas J. Lawley (Emory University, School of Medicine, Atlanta, GA) and grown as previously described (7). Human lung microvascular endothelial cells (HLMEC) were obtained from Clonetics (Baltimore, MD) and cultured in endothelial cell Basal Medium 2 (EBM-2) supplemented with EGM-2MV Single Quots (Clonetics, Baltimore, MD). Mouse embryonic endothelial cells (MEEC) were obtained and cultured as previously described (26).

Real-time RT-PCR. Real-time RT PCR assay was performed as previously described (12). Data are expressed as mean fold change ± standard deviation of at least three independent experiments. Primers used for real-time RT-PCR are available on request.

Apelin gene transcription was measured by quantifying pre-mRNA as previously described (30). Apelin pre-mRNA was measured by real-time PCR using the following intron-exon primers: upstream GTGAGTAGTAGGTCTCGGTTT targeted at intron 1 and downstream TTGCCGTCTTCCAGCCCATT targeted at exon 2. Apelin pre-mRNA expression was normalized to GAPDH expression measured by real-time PCR using the following primers: upstream GAAGGTGAAGGTCGGAGT targeted at exon 2 and downstream GAAGATGGTGATGGGATTTC targeted at exon 4.

siRNA transfection. Synthetic small interfering RNA (siRNA) targeting the human BMPR2 or ACVR2 mRNAs, and control siRNAs were purchased from Dharmacon Research (Lafayette, CO) and transfected into HMEC-1 cells using DharmaFECT-1 transfection reagent according to manufacturer’s recommendation. After transfection, cells were starved for 24h then treated with BMPs for 24h before lysis.
Sequences of siRNA used are available on request.

**Proliferation assay.** MECEs were seeded in DMEM 5% FCS at a density of 5000 cells/well in 96-well plates. The next day, cells were starved in serum-free DMEM for 24h before being stimulated with BMPs and exposed or not to hypoxia (1% O2). After 24 h, proliferation was measured by BrdU incorporation assay using Cell proliferation Elisa, BrdU kit (Roche Diagnostics) according to the manufacturer’s protocol.

**Recombinant adenoviruses construction** The adenovirus vector containing the complete coding sequence for the human apelin (Ad.APLN) was constructed according to He et al (18). The human SMAD7 expressing adenovirus was a gift from MJ Goumans (47).

**Statistical Analysis.** Two-way ANOVA with time as a repeated measure was used to determine time and treatment differences between the BMP treated and control in the cultured cells. Other multiple comparisons used one way ANOVA, followed by Student Newman–Keuls post hoc test. When two groups were compared, a non parametric Mann-Whitney test was used. Statistical significance was assumed at $P<0.05$. 

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**Results**

**BMPs down-regulate apelin in microvascular endothelial cells.**

DNA microarray experiments were performed to identify BMP target genes in HMEC-1 stimulated for 7 h or 24 h by 10ng/mL of BMP4. Among the modulated genes, including *ID1*, *ID2* and *ID3*, which were markedly up-regulated (data not shown), we observed a strong down-regulation of apelin expression by BMP4 at both 7 h and 24 h of stimulation. We confirmed, by real-time PCR, the down-regulation of apelin expression in HMEC-1 cells treated by 10ng/mL of BMP4 (Fig. 1A).

Apelin mRNA levels were also decreased by other members of the BMP family of cytokines, BMP7 and BMP9, similarly to BMP4 (Fig. 1A). We found that 4h after stimulation and for at least 24h, BMP7 and 9 inhibited by 60% and by up to 96% the mRNA expression of apelin, respectively. Similar results were obtained with BMP10 (data not shown).

Apelin expression was measured in primary human lung microvascular endothelial cells (HLMEC) after 24h of BMP treatment (50ng/mL). BMP4 and BMP7 inhibited apelin expression by 50% whereas BMP9 inhibited apelin expression for more than 96 % (Fig. 1B).

**Apelin down-regulation by BMPs is mediated by BMPR2 and the SMAD pathway**

We knocked-down the expression of *BMPR2* by using a *BMPR2*-targeting siRNA. BMP down-regulation of apelin expression is strongly decreased, but not abolished, in cells transfected with BMPR2-directed siRNA compared with those transfected with non-specific siRNA, and with cells transfected with an ACVR2A directed siRNA (Fig.2A), demonstrating that the down-regulation of apelin expression by BMP is mainly mediated by BMPR2. BMP down-regulation of apelin expression is also decreased in cells transfected with both BMPR2 and ACVR2A siRNAs (Fig.2A), showing that there is no functional rescue between the two
receptors. ACVR2B siRNA did not modify BMP9 apelin mRNA down-regulation (data not shown).

The canonic BMP signaling pathway involves SMAD phosphorylation of Receptor-Smads (R-Smads) and of the co-Smad, SMAD4. SMAD4 knock-down by siRNA resulted in an attenuated apelin down-regulation (Fig 2B). The inhibitory SMAD7 competes with R-Smad for the activated type I receptor binding site, and is therefore a signal transducing inhibitor.

We infected cells with a SMAD7 expressing adenovirus and observed a complete suppression of the apelin down-regulation by BMP9, and even a slight increase in basal apelin mRNA expression (Fig. 2C). This shows that BMP9 inhibits apelin expression via the SMAD pathway and that even in unstimulated conditions, the basal SMAD activity, possibly due to the presence of BMP ligands in the culture medium, inhibits apelin expression.

Molecular mechanism of apelin down-regulation by BMPs

In order to determine which step was involved in the BMP-induced apelin down-regulation, we inhibited DNA transcription or mRNA translation by pre-treating cells with actinomycin D or cycloheximide, respectively, before BMP stimulation. In actinomycin D-treated cells, we did not observe the down regulation of apelin expression in response to BMP, showing that BMP9 does not act on the stability of the apelin mRNA (Fig. 3A). After cycloheximide treatment, BMP still induced a decrease of apelin mRNA levels, although attenuated. Altogether, these results suggest that apelin inhibition by BMPs relies on a transcriptional mechanism and is in a large part independent of de novo synthesized proteins.

We analysed the transcriptional activity of the apelin gene by measuring its pre-mRNA expression by real-time PCR using primers that hybridize to sequences found in introns and, therefore, specifically recognize pre-mRNA (genomic DNA was removed during RNA
processing to avoid binding of primers). After 5h of BMPs treatment, pre-mRNA levels were
strongly reduced, showing that apelin transcription is inhibited by BMP stimulation (Fig. 3B).
We characterized the apelin promoter activity in response to BMP by transfecting HMEC-1
with luciferase reporter constructs under the control of fragments of the human apelin
promoter. None of the constructs tested, including one containing 2500bp of the proximal
promoter, responded to BMP stimulation (data not shown). We also tested the hypothesis that
the transcriptional down regulation of apelin by BMPs is due to the RNA degradation by a
microRNA. However by inhibiting the processing enzyme DROSHA with a DROSHA-
targeting siRNA, we did not observe any difference between BMP-treated and untreated cells
(data not shown). Thus, BMP downregulation of apelin expression involves a transcriptional
step, but its precise mechanism remains elusive.

Enforced expression of Apelin restored hypoxia-induced endothelial cell proliferation
inhibited by BMPs
We performed BrdU incorporation assay in endothelial cells treated with BMP4, BMP7 and
BMP9, exposed or not to hypoxia (1% O2). When endothelial cells were exposed to hypoxia
for 24h, BrdU incorporation was significantly increased. This effect was partially abolished in
the presence of BMPs (Fig 4A). Since, we demonstrated that apelin participates in hypoxia-
induced endothelial cell proliferation (13), we hypothesized that down-regulation of apelin
expression by BMPs inhibits the hypoxia-induced cell proliferation. Indeed, when we over-
expressed apelin by using an adenovirus vector we observed an increased BrDU incorporation
rate showing that inhibition of hypoxia-induced endothelial cell proliferation by BMPs is no
more detectable (Fig. 4B). Determination of apelin expression levels revealed that hypoxia-
induced apelin expression is reduced when cells are treated with BMPs (Fig. 4C).
Altogether, these results demonstrated that BMPs inhibit hypoxia-induced endothelial cell proliferation, at least in part, by inhibition of apelin expression.
Our results show that basal apelin mRNA expression is strongly inhibited at the transcriptional level in the HMEC-1 cell line and in lung microvascular endothelial cells by BMP 4, 7 and 9. This inhibition takes place at the transcriptional level, since BMPs decrease apelin pre-mRNA levels, and the inhibitory effect does not depend on mRNA stability and does not require, for a major part, de novo protein synthesis. The BMP9 effect was partially abrogated by knocking down the expression of \textit{BMPR2}, but not by depletion of the \textit{ACVR2A} receptor, which is partially redundant for some signal transducing pathways. Indeed, Yu et al showed that after BMPR2 inactivation, the BMP signal is transmitted through \textit{ACVR2}, but it is not the case for apelin down regulation (51). Blocking SMAD signaling by adenoviral overexpression of SMAD7 also totally suppressed the inhibitory effect of BMPs on apelin, clearly showing that only the SMAD pathway is responsible for the inhibition.

A 2500bp fragment of the apelin promoter including the 5’ untranslated region of the mRNA failed to reproduce the response to BMP signals although it contained several putative SMAD binding elements (25), suggesting that putative responsive elements would be located elsewhere in the gene, or that another mechanism is implicated. Since SMAD proteins were shown to promote primary miR-21 processing by acting within the \textit{DROSHA} complex (10), we tested the hypothesis of a microRNA mediated inhibition of apelin expression by BMP using the knocking-down of \textit{DROSHA}, in order to inhibit the processing of a putative primary miRNA which might have decreased \textit{APLN} mRNA levels. The absence of effect observed with the \textit{DROSHA} siRNA on apelin down-regulation by BMP does not favour this hypothesis. Therefore, BMPs acts on apelin expression through the BMPR2-SMAD signaling pathway on apelin transcription, but the comprehensive mechanism leading to expression inhibition remains elusive.
Our results are at discrepancy with those of Alastalo et al who showed that BMP2 induces a transcriptional complex associating PPARγ and β-catenin, which in turn induces APLN transcription, through BMPR2 signaling (1). Our results are difficult to reconcile with these findings, although we didn’t use BMP2, but we observed the APLN mRNA suppression with BMP4, 7 and 9.

As an example of the relevance of our results in pathological conditions, we found an inhibitory effect of BMPs on hypoxia-induced proliferation of endothelial cells, which correlated with the inhibition of hypoxia-induced apelin expression by BMP in MEEC. Such an inhibitory effect on EC growth has been shown by David et al with BMP9 (9), and BMP9 was also shown to be a vascular quiescent factor, able to inhibit angiogenesis in in vivo models (8). The inhibition of EC proliferation by BMP and its reversion by APLN enforced expression suggest that the BMP-apelin interaction could play a role in vascular homeostasis, in particular during hypoxia. Indeed, during hypoxia, complex results have been obtained in vitro and in vivo on the BMP signaling pathway in vascular cells of the lung. BMP2 and BMP4 were shown to be increased by hypoxia in the early phase of rat in vivo models of hypoxia (41, 45), but BMP signaling is attenuated in the lung vessels during hypoxia models at later stages in rat models (41). It has also been shown that BMP signaling is decreased under hypoxia at the level of target genes transcription by interaction with CtBP1 in cultured human pulmonary VSMC (48). BMP antagonists, such as gremlin and chordin were also shown to be increased during hypoxia (6, 20). We previously demonstrated that apelin expression is increased by hypoxia (13) and we show here that this increase is partially suppressed by BMP. Therefore, pathological conditions where BMP expression is increased are the most likely to inhibit APLN expression, and inversely, those where BMP is decreased, as during experimental and human pulmonary arterial hypertension (PAH), APLN expression is likely to increase, as observed by SMAD7 inhibition in our study.
Our results differ from results obtained previously by De Jesus Perez et al. who showed that BMP2 promotes pulmonary artery endothelial cell survival, proliferation and motility (11). Indeed, each BMP member seems to have specific biological effects depending on the physiological conditions and the cell type. The BMP receptor involved in the signal transduction also determines the targeted genes (46).

Strong arguments support the role of BMP signaling in PAH development even if the sequence of the cellular and molecular events is still not clear. Mutations of the BMPR2 gene observed in the human heritable form of PAH lead to haplo-insufficiency (31), and BMP pathway activity is reduced in the pulmonary vasculature of patients suffering from pulmonary hypertension with or without mutations on BMPR2 (2, 50). Mice heterozygous for the Bmpr2 gene KO are more susceptible to PAH inducers (14, 38) and BMP signaling is decreased in two models of rat PAH (29). The finding that BMP signaling inhibits apelin expression can be important in the context of PAH associated with BMPR2 mutations. It is indeed possible that, in such conditions, APLN expression by pulmonary endothelial cells is abnormally increased, due to a weakened BMP inhibition. We can speculate that direct target genes of the BMP pathway such as APLN are good candidates to participate in the mechanisms by which BMPR2 deleterious mutations define a subclass of patients developing the disease earlier and with a more severe hemodynamic condition than non-carriers (15, 40).

A genetically deficient BMP pathway would blunt the apelin down-regulation, leading to increased remodeling by apelin, as can be expected from results obtained on the carotid ligation model by Kojima et al, who showed that apelin null mice have less severe artery lesions, since they have significantly less neointima and a smaller mean total vascular area than wild type mice (24). These data contrast with the angiotensin II model of atherosclerosis in ApoE-KO mice, where mice with a double inactivation of the ApoE and apelin genes display more severe lesions than mice with a functional apelin gene. These conflicting results
emphasize the dual function of apelin on EC, and on SMC. In the human lung, apelin expression is restricted to the endothelium of small vessels (23) while its receptor APLNR (42) is found in both endothelial and smooth muscle cells of pulmonary vessels (22, 23), suggesting that apelin secreted from the endothelium could exert autocrine and paracrine effects on endothelial and the neighbouring smooth muscle cells. Apelin exerts contrasted effects on vascular tone, depending on the target cells reached. In rats, apelin decreases mean arterial pressure by inducing NO release from endothelial cells (5, 43) but stimulates contraction when it acts directly on smooth muscle cells (5, 17, 22, 43). Apelin has been found to induce the proliferation of vascular smooth muscle cells of mouse aortas (16). An increase in apelin expression may thus lead to an increase of both vascular tone and SMC proliferation and, consequently, might contribute to the medial hypertrophy observed in pulmonary hypertension. However, these results are discordant with those of Alastalo et al, since they reported that conditioned medium of apelin depleted EC increases PASMC proliferation (1). These cell-specific effects could be responsible for the contrasted results obtained in vivo with apelin KO mice. It has been shown that APLN disruption in mice worsens the pulmonary vascular proliferative lesions induced by hypoxia, and this can be due to the decreased NO release from endothelial cells when apelin is absent (4). But inversely, apelin disruption improves the carotid lesions in a model of carotid ligature in mice as mentioned above (24).

In summary, we report that BMPs inhibit apelin expression in endothelial cells through a BMPR2-SMAD dependent mechanism. Moreover, we determined that BMP inhibition of apelin expression regulates hypoxia-induced endothelial proliferation.

Altogether these results uncover an important link between a signaling pathway involved in PAH and a peptide regulating vascular homeostasis. A genetically deficient BMP pathway, as observed in the presence of a BMPR2 or an ACVRL1 monoallelic hypomorphic
mutation, might modulate the interaction between BMP and apelin and increase the vascular remodeling of pulmonary small arteries.
Acknowledgments

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Legends to figures

Fig. 1. BMPs down-regulate apelin in microvascular endothelial cells. (A) HMEC-1 were stimulated with 10ng/mL BMP4, 7 or 9 for 4, 7 or 24h. The levels of apelin mRNA were determined by real-time RT-PCR analysis. The relative apelin expression levels were calculated as the ratio of apelin expression in BMP-treated cells to those of untreated cells for each time point, both, normalized to RPL32 levels. Results are mean ± SD. values of three independent experiments. *, p<0.05 relative to untreated cells. (B) HLMECs were stimulated with 50ng/mL BMP4, 7 or 9 for 24h. The relative apelin expression levels were calculated as the ratio of apelin expression in BMP-treated cells to those of untreated cells, both normalized to RPL32 levels. Results are mean ± SD values of three independent experiments, * p<0.05 relative to untreated cells.

Fig. 2. Apelin down-regulation by BMP9 is mediated by the BMPR2 and SMAD pathway. (A) HMEC-1 were transfected with a non specific (si CT), a BMPR2 specific siRNA (si BMPR2), or ACVR2A siRNA (siACVR2A), or transfection agent only (T0). After transfection, cells were starved overnight, stimulated with BMP9 (10ng/mL) for 24h and then total RNA was extracted. The relative apelin expression levels were calculated as the ratio of apelin expression in experimental cells to those transfected with siRNA-CT cells, normalized to GAPDH levels. Results are mean ± SD. Values of 3 independent experiments. *, p<0.05 relative to BMP9 treated cells transfected with siCT. No significant (NS) difference between siBMPR2 and siACVR2A plus siBMPR2 transfected cells treated with BMP9.

(B) HMEC-1 were transfected with a non specific (siCT) or a SMAD 4 specific siRNA (siSMAD4). After transfection, cells were starved overnight, and stimulated with BMP 9 (10ng/ml) for 24 h and total RNA was extracted. The relative apelin expression levels were calculated as the ratio of apelin expression in BMP9 treated cells to those of untreated cells,
both normalized to GAPDH. Results are mean +/- SD values of 6 independent experiments.

**, p<0.01, relative to BMP9 treated cells transfected with siCT.

(C) HMEC-1 were infected with adenoviruses coding for beta-galactosidase (AdlacZ) or SMAD7 (AdSMAD 7) for 48 h. After infection, cells were starved overnight, stimulated with BMP9 (10ng/ml) for 24 h and then total RNA was extracted. The relative apelin expression levels were calculated as the ratio of apelin expression in BMP9 treated cells to those of untreated cells, both normalized to GAPDH levels. Results are mean ± SD. values of three independent experiments. p<0.05 AdSMAD7 vs AdLacZ infected treated (*) or not treated ($) with BMP9. No significant (NS) difference between AdSmad7 infected cells treated or not with BMP9.

Fig. 3. Apelin down-regulation by BMPs is dependent on a transcriptional mechanism.

(A) HMEC-1 cells were pre-treated or not with actinomycin D (ActD) (5μg/ml) or cycloheximide (CHX) (5μg/ml) for 15 min before being stimulated with BMP 9 (10 ng/mL) for 24 h. The relative apelin expression levels were calculated as the ratio of apelin expression in BMP9-treated cells to those of control cells, both normalized to GAPDH levels. Results are mean ± SD values of 3 independent experiments. ***, p<0.0001; ** p<0.01 relative to control untreated cells; $$, p<0.01 for BMP9+CHX treated cells vs CHX treated cells comparison; §§, p<0.01 for BMP9+ActD treated cells vs BMP9 treated cells comparison. No statistically significant difference was found between control BMP9 treated cells and BMP9+CHX treated cells, and between ActD treated cells and BMP9+ ActD treated cells.

(B) HMEC-1 were treated with 10ng/mL BMP4 and BMP7 for 5h. Nuclear RNAs were isolated and analysed by real-time PCR using primers that hybridize to intron 1 and exon 2 and detect only the apelin pre-mRNA. Apelin pre-mRNA expression was normalized to GAPDH mRNA expression. Results are mean ± SD. values of 3 independent experiments. *, p<0.05 relative to control cells.
Fig. 4 BMPs inhibit hypoxia-induced endothelial cell proliferation through the inhibition of apelin expression. (A) BrdU incorporation in MEECs treated or not with BMP4, 7 or 9 at 50 ng/ml and exposed or not to hypoxia (1% O2) for 24h. Results represent means ± SD of 3 independent experiments. *, p<0.05 relative to hypoxia alone. (B) BrdU incorporation in MEECs infected with a control adenovirus (AdGFP) or an adenovirus coding for human apelin (AdAPE), treated or not with BMP 4, 7 or 9 (50 ng/mL) and exposed or not to hypoxia (1%O2). Results represent means ± SD of 3 independent experiments. *, p<0.05 relative to normoxia. (C) MEECs were treated with BMP4 or BMP9 (50ng/mL) and exposed or not to hypoxia (1% O2) for 24h. The levels of apelin mRNA were determined by real-time RT-PCR analysis. The relative apelin expression levels were calculated as the ratio of apelin expression in experimental cells to those of normoxic untreated cells, both normalized to RPL32 levels. Results are mean ± SD values of three independent experiments. *, p<0.05 relative to normoxic untreated cells.

References


Fig. 1: BMPs downregulate Apelin expression in microvascular endothelial cells.
Fig.2: Apelin downregulation by BMP9 is BMPR2- and SMAD-dependent
Fig. 3: Apelin downregulation by BMP2 and 4 is transcriptional and indirect
Fig. 4 BMPs inhibit hypoxia-induced endothelial cell proliferation through the inhibition of apelin expression.