Antenatal Administration of Betamethasone Promotes Closure of Preterm Ductus Arteriosus via Intimal Thickening Formation

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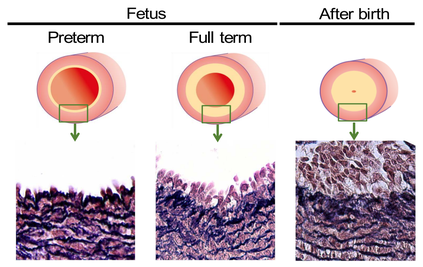
BACKGROUNDS

- Antenatal betamethasone (BTM) administration is widely accepted to reduce respiratory distress syndrome. In addition, some observational studies indicate that BTM decreases prevalence of patent ductus arteriosus (PDA) in preterm infants.

Author	Study period	Inclusion criteria	PDA reduction rate
Morales et al.	1986-1988	Gestational age 26-34w (n=165)	67%
Amorim et al.	1997-1998	Gestational age 26-34w (n=218)	73%
Elimian et al.	1990-1997	Birth weight 500-1750g (n=527)	44%
Been et al.	2001-2003	Gestational age <32w (n=121)	44%

- Closure of the ductus arteriosus (DA) requires morphological remodeling, i.e., intimal thickening (IT) formation. However, the role of BTM in IT formation of the preterm DA has not been reported.



① Subendotherial edema
Disruption of internal elastic lamina
② Proliferation and migration of smooth muscle cells

Intimal thickening(IT)

(Yokoyama U, et al. J Clin Invest., 2006.)
(Yokoyama U, et al. J Smooth Muscle Res., 2010.)

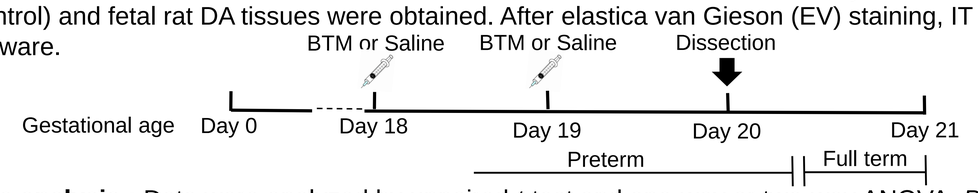
OBJECTIVE

To examine the role of antenatal BTM in DA IT formation.

MATERIALS AND METHODS

- **Tissues :** Wistar fetal rats were obtained from time-pregnant mother (SLC).
- **Cells :** Smooth muscle cells of rat DA (DASMCs) and smooth muscle cells of rat aorta (ASMCs) on day 20 of gestation were obtained by primary culture.
- **Reagents :** Betamethasone sodium phosphate (WAKO) , Actinomycin D (Sigma-Aldrich) , Art3-targeted siRNA (Applied Biosystems) , and anti-p-FAK, anti-p-paxillin antibodies (Santa Cruz Biotechnology) were used. A BrdU Cell Proliferation Assay Kit (Sigma-Aldrich) was used.
- **Expression of mRNAs and proteins:** qRT-PCR using SYBR Green and Immunofluorescent Staining were performed.
- **Microarray analysis :** SurePrint G3 Rat GE v2 8x60K Microarray (Agilent).
- **Scratch assay :** Half of DASMCs on cell culture dish were shaved off and migration area was measured at 24-72 h after BTM stimulation with or without *Art3*-targeted siRNAs transfection.

- **Antenatal BTM administration :** Maternal rats were administered intravenously with BTM 0.4 mg/kg or normal saline (control) and fetal rat DA tissues were obtained. After elastica van Gieson (EV) staining, IT area were measured by image J software.



- **Data analysis :** Data were analyzed by unpaired *t*-test and one-way or two-way ANOVA. *P* <0.05 was considered significant.

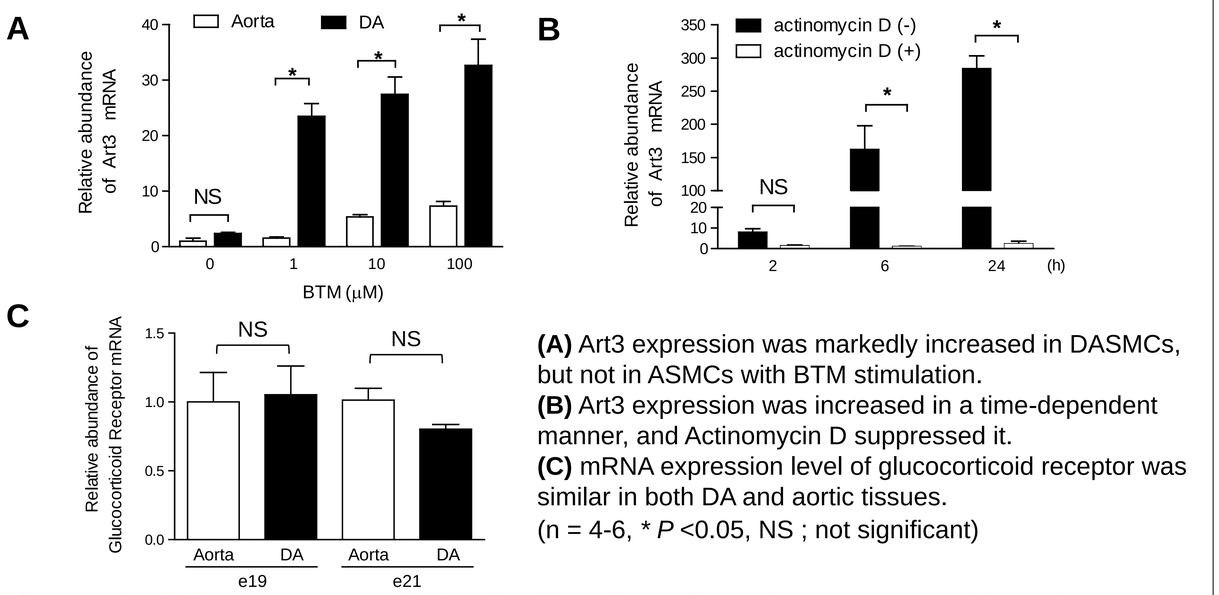
RESULTS

Figure 1. Microarray data revealed BTM-induced genes in rat DASMCs

DASMCs(day 20 of gestation) were stimulated by BTM 100 mM
↓
58,717genes were detected
↓
109 probe sets were > 2.0-fold increased by BTM stimulation. (*P* <0.05)

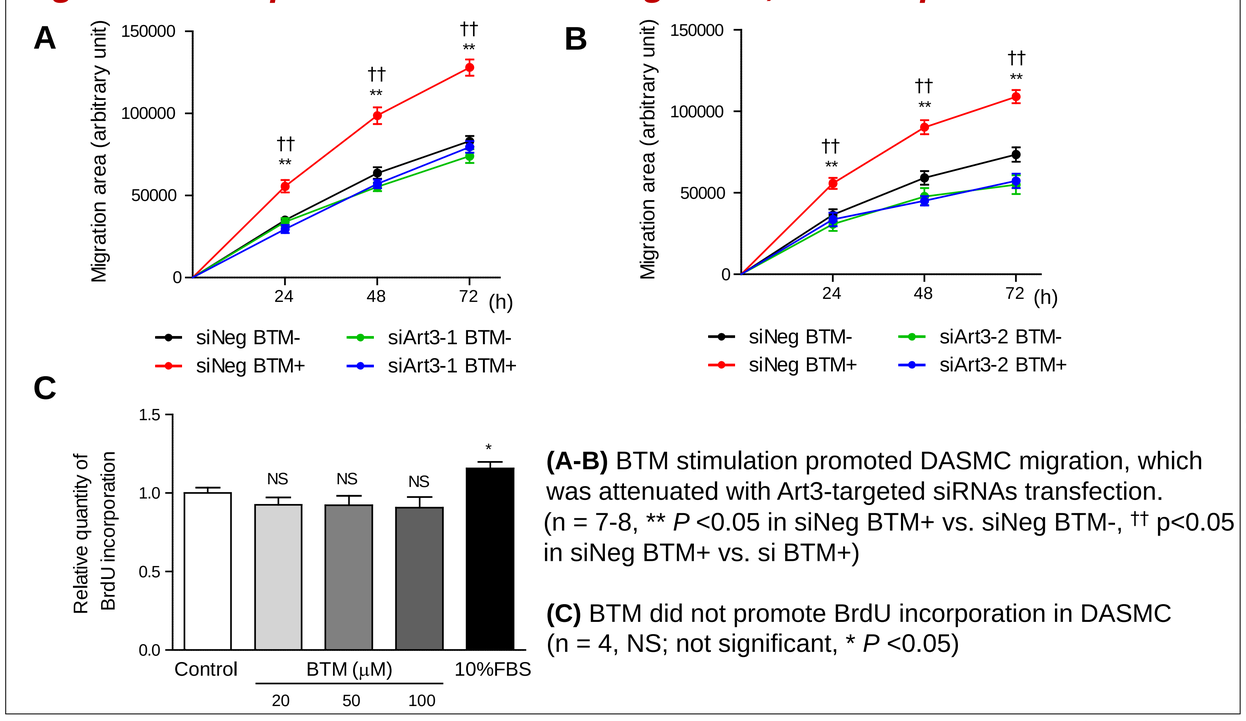
Gene Symbol	mRNA description	Fold change (BTM/Control)
Art3	ADP-ribosyltransferase 3	11.1
Scgb2a2	Secretoglobin, family 2A, member 2	8.40
Rapgef5	Rap guanine nucleotide exchange factor 5	4.06
Xdh	Xanthine dehydrogenase	2.50
Lpar1	Lysophosphatidic acid receptor 1	2.38
⋮	⋮	⋮

Figure 2. BTM increased Art3 mRNA transcription in rat DASMCs.



(A) Art3 expression was markedly increased in DASMCs, but not in ASMCs with BTM stimulation.
(B) Art3 expression was increased in a time-dependent manner, and Actinomycin D suppressed it.
(C) mRNA expression level of glucocorticoid receptor was similar in both DA and aortic tissues.
(n = 4-6, * *P* <0.05, NS ; not significant)

Figure 3. BTM promoted DASMC migration, but not proliferation.

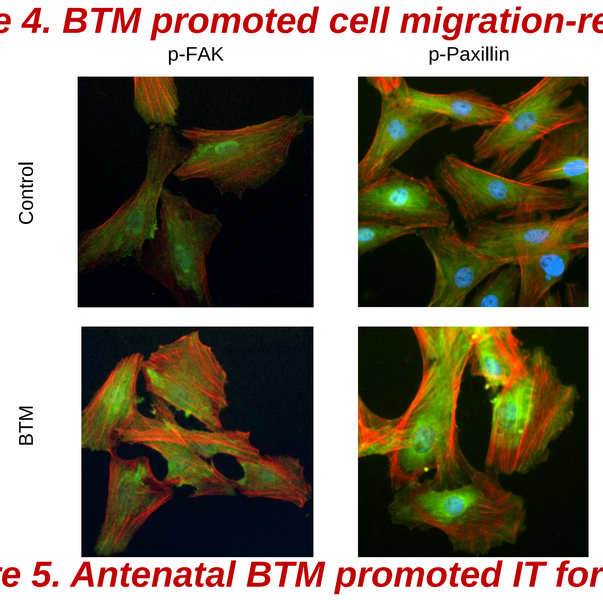


(A-B) BTM stimulation promoted DASMC migration, which was attenuated with Art3-targeted siRNAs transfection.
(n = 7-8, ** *P* <0.05 in siNeg BTM+ vs. siNeg BTM-, †† p<0.05 in siNeg BTM+ vs. si BTM+)

(C) BTM did not promote BrdU incorporation in DASMC
(n = 4, NS; not significant, * *P* <0.05)

RESULTS

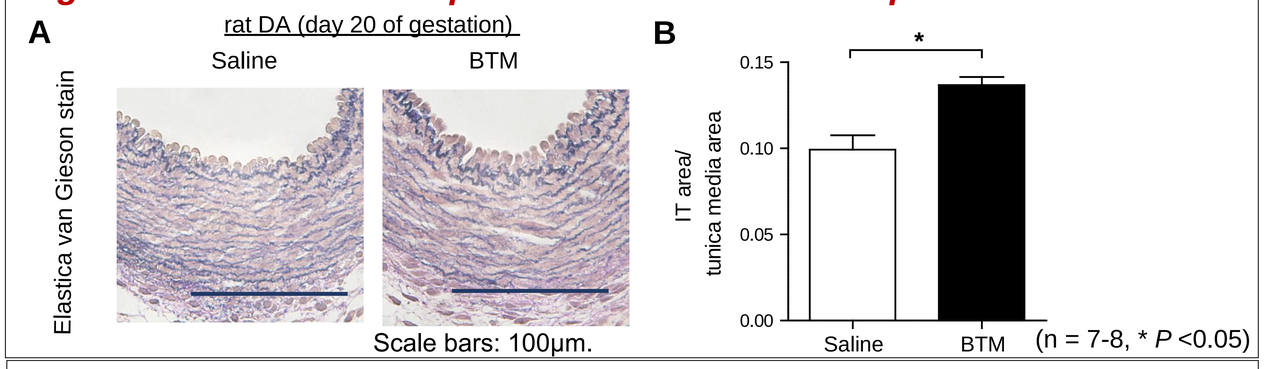
Figure 4. BTM promoted cell migration-related proteins in rat DASMCs.



Green and Red color indicates positive immunoreaction for p-FAK or p-paxillin and F-actin, respectively.

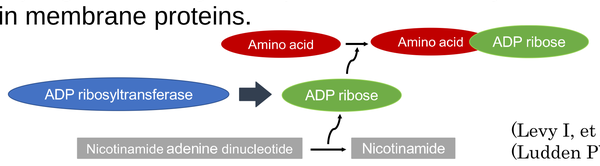
Both p-FAK and p-paxillin were highly expressed with BTM stimulation.

Figure 5. Antenatal BTM promoted IT formation in preterm rat DA.



About Art3 (ADP-ribosyltransferase3)

- ADP ribosyltransferases (ARTs) regulate endogenous protein functions by attaching ADP-ribose to specific amino acid residues in membrane proteins.



(Levy I, et al. FEBS Lett., 1996)
(Ludden PW, et al. Mol Cell Biochem., 1994)

- Art3 is a member of the ART family, of which biological function is almost unkown.

(Okazaki I, et al. Annu Rev Nutr., 1999)
(Glowacki G, et al. Protein Sci., 2002)

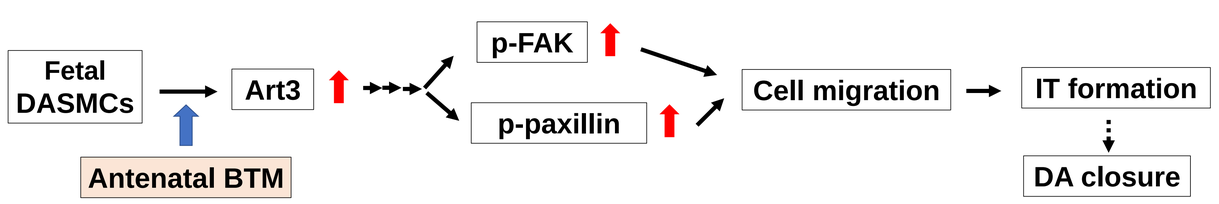
- Art3 expression is enhanced by glucocorticoid in rat cardiomyocytes.

(Olivier M. Genomics., 2007)

CONCLUSIONS

Antenatal BTM administration may contribute to DA IT formation through Art3-mediated DASMC migration.

Proposed mechanism of BTM induced DA remodeling



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