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Regulatory expression of bone morphogenetic protein-6 system in aldosterone production by human adrenocortical cells

Kenichi Inagaki, Fumio Otsuka *, Jiro Suzuki, Hiroyuki Otani, Masaya Takeda, Yoshihiro Kano, Tomoko Miyoshi, Misuzu Yamashita, Toshio Ogura, Hirofumi Makino

Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama City, 700-8558, Japan

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Abstract

Bone morphogenetic protein-6 (BMP-6) enhances aldosterone production by upregulating angiotensin II (Ang II)-to-MAPK pathway. Here we investigated effects of Ang II and potassium on the BMP system in human adrenocortical H295R cells. BMP-6 transcription was transiently downregulated by treatments with Ang II and potassium. Aldosterone also decreased BMP-6 expression at a high concentration. Chemical inhibitions of transcription and translation abolished the transient reduction of BMP-6, suggesting that destabilization of BMP-6 mRNA was hardly involved while new protein synthesis was possibly mediated in this mechanism. However, BMP-6 protein was stably detected during the exposures of Ang II and potassium. Notably, Ang II, potassium and aldosterone decreased mRNA levels of follistatin that extracellularly neutralizes bioactivities of activins and BMPs although the BMP-6 receptor expression was unaffected. Given the maintenance of bioavailable BMP-6 protein and the receptor expression in adrenocortical cells, endogenous BMP-6 may be a key autocrine modulator for aldosterone production.

Keywords: Adrenal cortex; Angiotensin II; Aldosterone; Bone morphogenetic protein (BMP); Follistatin; Potassium (K); H295R cell

1. Introduction

Production of aldosterone occurs in the adrenal glomerulosa, which is systemically regulated by angiotensin II (Ang II), potassium (K) and adrenocorticotropin (ACTH) [1,2]. In the presence of these aldosterone stimulators, steroidogenesis in the adrenal cortex is further governed by local autocrine and/or paracrine regulators [3]. In this respect, we reported the presence of a functional BMP and activin system complete with ligands including BMP-6, activins and these receptors in the human adrenocortical cell line H295R [4]. BMPs were originally identified as the active components in bone extracts capable of inducing bone formation at ectopic sites. A variety of physiological BMP actions in many endocrine tissues including the ovary [5–7], pituitary [8,9], thyroid [10] and adrenal [4,11] have been uncovered to date.

In human adrenocortical cells BMP-6 stimulates aldosterone production with increased expression of rate-limiting steroidogenetic enzymes [4]. BMP-6 enhances Ang II-induced but not ACTH-induced aldosterone production, whereas activin enhances ACTH-induced aldosterone production. Follistatin, which enables to bind activins [12] and BMPs [13–16] suppresses basal and ACTH-induced aldosterone production. Hence, BMP-6 is preferentially involved in aldosterone production by modulating Ang II signaling in human adrenal cortex, whereas activin is linked to aldosterone synthesis by modulating the ACTH–cAMP–protein kinase A (PKA) signaling [4].

Abbreviations: ACD, actinomycin D; ACTH, adrenocorticotropin; ALK, activin receptor-like kinase; ActRII, activin type II receptor; Ang II, angiotensin II; BMP, bone morphogenetic protein; BMPRII, BMP type II receptor; CHX, cycloheximide; ERK, extracellular signal-regulated kinase; FSK, forskolin; MAPK, mitogen-activated protein kinase; TGF-β, transforming growth factor-β.

* Corresponding author. Tel.: +81 86 235 7235; fax: +81 86 222 5214.
E-mail address: fumiotsu@md.okayama-u.ac.jp (F. Otsuka).

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Table 1
Primer settings for RT-PCR analysis

	Sense primer	Antisense primer	Product size (bp)	Genbank accession no.
BMP-6	5′-CAGGAGCATCAGCACAGAG	5′-TTCATGTGTGCGTTGAGTGG	521	NM_001718
	5′-CAGGAGCATCAGCACAGAG	5′-CCTCCTTGCAGGCTGTTTTC	402	
ALK-2	5′-ACTATCGAAGGGCTCATCACCA	5′-GGTCCCAAATATCTCTATGTGC	483	Z22534
ALK-3	5′-ACATCAGATTATTGGGAGCC	5′-TGTAACAAAAGCAGCTGGAG	510	NM_004329
ActRII	5′-GGGAAAATGGGAGCTGCTGC	5′-CCTGTACACCCAAAATGCAC	492	D31770
Follistatin	5′-GCTGGGCAGATCTATTGGATT	5′-CATTGTCACTGGCACAGACAG	188	BC004107
RPL19	5′-CTGAAGGTGAAGGGGAATGT	5′-AAGTCTTGATGATCTCCTCC	190	NM_000981

Further molecular approaches have elucidated that BMP-6 augments Ang II-induced aldosterone production through upregulating mitogen-activated protein kinase (MAPK) signaling via the receptor complex composed of type I receptors including activin receptor-like kinase (ALK)-2 and/or ALK-3, and activin type II receptor (ActRII) [17]. However, the physiological significance of endogenous BMP system in the adrenal cortex is still poorly understood. We therefore investigated the regulation of BMP-6 expression and the changes of BMP receptor expression in a process of aldosterone production induced by Ang II and potassium.

2. Materials and methods

2.1. Reagents and supplies

A 1:1 mixture of Dulbecco's Modified Eagle's Medium/Ham's F12 medium (DMEM/F12), penicillin–streptomycin solution, angiotensin II acetate salt (Ang II), adrenocorticotropic hormone (ACTH) human fragment 1–24, forskolin (FSK), actinomycin D, cycloheximide, d-aldosterone, and spironolactone were purchased from Sigma-Aldrich Co. Ltd. (St. Louis, MO). Recombinant human BMP-6 was purchased from R&D Systems (Minneapolis, MN). NuSerum™ and insulin–transferrin–sodium selenite Plus (ITS+™) were from BD-Falcon (Bedford, MA).

2.2. Cell culture and RNA extraction

The NCI-H295R human adrenocortical cell line was obtained from American Type Culture Collection (Manassas, VA). H295R cells were cultured in DMEM/F12 medium containing 4 mM of potassium, 2.5% NuSerum™, 1% ITS+™ supplements and antibiotics (penicillin and streptomycin). The cells were cultured at 37 °C under a humid atmosphere of 95% air/5% CO_2 as previously reported [18]. A human granulosa cell line KGN was kindly provided by Drs. Masatoshi Nomura and Hajime Nawata (Kyushu University, Japan). H295R cells were grown in 12-well plates to ~80% confluence, then the medium was replaced with low-serum medium containing 0.3% NuSerum™. The cells were treated with either alone or combinations of the reagents including Ang II, potassium, ACTH, forskolin, aldosterone, spironolactone, actinomycin D, and cycloheximide at indicated concentrations. After culture for 3 to 48 h, the medium was removed and total cellular RNA was extracted by isothiocyanate–acidphenol–chloroform methods using TRIzol® (Invitrogen Corp., Carlsbad, CA) and quantified by measuring absorbance at 260 nm and stored at −80 °C until assay.

2.3. Aldosterone measurement

To assess the effects of BMP-6 on aldosterone production, monolayered cells (~80% confluency) were precultured in 24-well human fibronectin-coated plates (Biocoat®, BD-Falcon) and after 24-h preculture, the medium was replaced with fresh medium containing 0.3% NuSerum™ with Ang II, potassium, ACTH, and forskolin in combination of BMP-6 at indicated concentrations. H295R cells were then cultured for another 48 h and the accumulated levels of aldosterone in the conditioned media were determined by radioimmunoassay using SPAC-S aldosterone kit (T.F.B. Co., Tokyo, Japan).

2.4. RT-PCR and quantitative real-time PCR analysis

Oligonucleotides used for RT-PCR were custom-ordered from Kurabo Biomedical Co. (Osaka, Japan). PCR primer pairs were

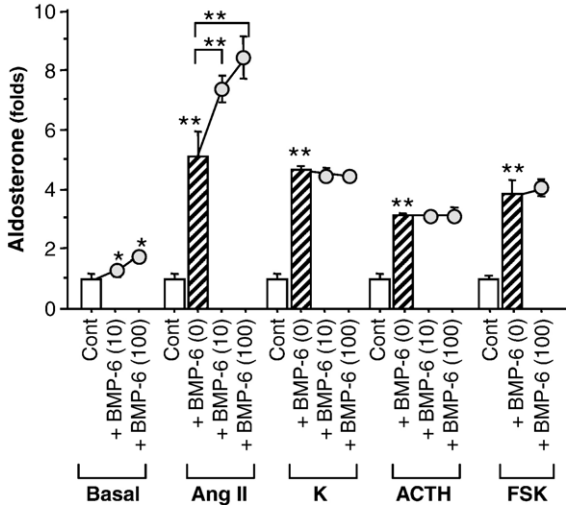

Fig. 1. Effects of BMP-6 on aldosterone production by H295R cells. After preculture of the cells, the medium was replaced with fresh medium containing 0.3% NuSerum™ and treated with Ang II (10 nM), potassium (K; 16 mM), ACTH (100 ng/ml) or forskolin (FSK; 10 μM) in combination with BMP-6 (ng/ml) for 48 h. Accumulated aldosterone levels in the conditioned media were determined by radioimmunoassay. All results are shown as mean±SEM of the data from at least three separate experiments, each performed with triplicate samples. *$P<0.05$ and **$P<0.01$ vs. control or between the indicated groups.