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Poly(ADP-ribose) polymerase-1 (PARP-1) transcriptionally regulates angiotensin AT2 receptor (AT2R) and AT2R binding protein (ATBP) genes

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ABSTRACT

The renin–angiotensin system (RAS) plays a crucial role in cardiovascular and neuronal (patho-) physiology. The angiotensin AT2 receptor (AT2R) seems to counteract the proinflammatory, prohypertrophic and profibrotic actions of the AT1 receptor. Recently, we identified a novel protein, termed "AT2R binding protein" (ATBP/ATIP) which seems essential for AT2R-mediated growth inhibition. Poly(ADP-ribose) polymerase-1 (PARP-1) can act as a nuclear integrator of angiotensin II-mediated cell signalling, and has been implicated in the pathogenesis of cardiovascular and neuronal disease.

In this study, promoters of human AT2R and ATIP1 were cloned and two transcriptional start sites in the ATIP1 promoter were identified whereas only one was detected in the AT2R promoter. Promoter assays indicated that the exon 1–intron 1 region of AT2R is necessary and sufficient for AT2R promoter activity. Inverse cloning experiments indicated that this regulatory region is a promoter but not an enhancer element implicating (a) further start site(s) in this region. Consistently, the exon 1–intron 1 region of AT2R was shown to tether the basal transcriptional machinery. Overexpression, pharmacological inhibition and ablation of PARP demonstrated that PARP-1 activates the ATIP1 gene but represses the AT2R on promoter and mRNA levels *in vitro*, and in brain tissue *in vivo*. Additional experiments indicated that AT2R activation does not modulate PARP-1 transcript levels but increases AT2R promoter activity, thereby creating a positive feedback mechanism.

Our results demonstrate that PARP-1 acts as novel node within the RAS network based on its ability to regulate downstream targets such as AT2R and its adapter protein ATBP.

1. Introduction

The renin–angiotensin system (RAS) constitutes one of the best characterised systems within mammalian physiology and pathophysiology. It is causally involved in a broad spectrum of diseases ranging from arterial hypertension, cardiac hypertrophy, diabetic nephropathy and stroke to cancer and inflammation [1–4]. The effects of the RAS are mainly mediated by two receptors, the angiotensin AT1 receptor (AT1R) and the angiotensin AT2 receptor (AT2R) [1]. Several groups have demonstrated that AT2R activation can counteract the vasoconstrictory, prohypertrophic and pro-proliferative effects of AT1R activation [1,5,6], in addition to its ability to mediate neuroprotective effects [7,8]. Nevertheless, the (patho)physiological role of AT2R is still controversial [9,10]. We recently postulated that the type of adapter protein recruited to the AT2R determines its cellular effects [11]. The AT2R can couple to certain G proteins [12,13] and the SH2 domain-containing phosphatase 1 (SHP-1) [14]. The physical interaction with the latter is probably related to the antiproliferative effects of the AT2R [9,15]. In contrast, recruitment of the promyelocytic zinc finger protein (PLZF) to the AT2R was shown to be associated with an increase in protein synthesis of cardiomyocytes and cardiac hypertrophy [16].

Our group was recently able to identify ATBP (AT2R binding protein) as a novel protein with the ability to bind to the cytoplasmic

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Abbreviations: RAS, renin–angiotensin system; AT1R, angiotensin AT1 receptor; AT2R, angiotensin AT2 receptor; PARP-1, poly(ADP-ribose) polymerase-1; SHP-1, SH2 domain-containing phosphatase 1; PLZF, promyelocytic zinc finger protein; ATBP, AT2R binding protein; ATIP, AT2 receptor interacting protein; MTSG, mitochondrial tumor suppressor gene 1; PAR, poly(ADP-ribose); MEFs, mouse embryonic fibroblasts; RLA, relative luciferase activity; ChIP, chromatin-immunoprecipitation; TBP, TATA box-binding protein.

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C-terminus of the AT2R [17]. ATBP is expressed in at least three different isoforms termed ATBP50, ATBP60 and ATBP135 [17]. ATBP50 plays a crucial role concerning the transport of the AT2R from the Golgi compartment to the cell membrane, and mediates the inhibitory effects on MAP kinases as well as the antiproliferative effects of the AT2R in neuroblastoma cells [17]. The ATBP gene was simultaneously cloned by two other groups who termed this gene AT2 receptor interacting protein (ATIP) [18,19] and mitochondrial tumor suppressor gene 1 (MTSG1) [20], respectively, and who also demonstrated the inhibitory effects of this protein on cell proliferation and MAP kinases.

ATIP1 (=ATBP50) is the major isoform since it was initially identified by the yeast two-hybrid screenings [17,18], is expressed ubiquitously [17,19] and constitutes the most abundant isoform, e.g. in brain, heart, breast and ovary [19]. ATBP can also function as a tumor suppressor gene [20] and very recently it was shown that a genetic copy number variant (CNV) affecting the ATBP gene locus as a deletion polymorphism is significantly associated with a decreased risk of breast cancer [21].

PARP-1 is an ubiquitous nuclear protein catalysing the attachment of poly(ADP-ribose) (PAR) polymers to several different acceptor proteins such as histones and RNA polymerase II [22,23]. PARP-1 is involved in pathophysiologies such as hypertension, stroke, neuronal injury, diabetes and inflammation [24] thereby contributing to similar disease states as the RAS. Consistently, animal experiments indicated that cardiac hypertrophy and also endothelial dysfunction induced by angiotensin II are absent in homozygous PARP-1 deficient mice [25,26]. Nevertheless, the regulatory mechanisms of this RAS–PARP interplay are largely unknown. Therefore, we analysed basal and PARP-mediated regulation of the AT2R and its interaction partner ATBP/ATIP on chromatin, promoter and mRNA levels.

2. Materials and methods

2.1. Cell culture

Human endothelial EA.hy926 cells were kindly provided by C.-J. Edgell (Chapel Hill, NC, USA) and cultured in high glucose DMEM (Invitrogen, Karlsruhe, Germany) supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin, 1 mM sodium pyruvate, 1% non-essential amino acids and 2% HAT (all from Invitrogen).

The human neuroblastoma cell line KELLY was a kind gift of the Physiology Department (Campus Mitte) of the Charité, Berlin, Germany. KELLY cells were cultured in RPMI (Invitrogen) supplemented with 10% FCS, 100 U/ml penicillin and 100 μg/ml streptomycin. For transfection experiments KELLY cells were cultured on plates coated with poly-L-lysine (0.005 mg/ml; Biochrom, Bremen, Germany). The human breast adenocarcinoma cell line MCF-7, a kind gift of G. Schönfelder (Toxicology Department, Charité), was cultured in high glucose DMEM supplemented with 10% FCS and 100 U/ml penicillin and 100 μg/ml streptomycin. Mouse embryonic fibroblasts (MEFs) were obtained from Z.-Q. Wang (Leibniz Institute for Age Research, Jena, Germany) and were grown in high glucose DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate and 0.2% beta-mercaptoethanol (Invitrogen).

All cell lines were grown in a humidified atmosphere of 5% CO_2 in air and at 37 °C.

Cellular total RNA was isolated using the RNA isolation kit from Macherey–Nagel (Düren, Germany).

For stimulation experiments the following small molecules were used: the non-selective PARP inhibitor 3-aminobenzamide (3-AB; Sigma, Hamburg, Germany; 10 mM (EA.hy926) or 30 mM (KELLY)), the AT2R agonist compound 21 (1 μM in water or PBS; a kind gift of Vicore Pharma, Gothenburg, Sweden), the AT1R blocker irbesartan (10 μM in DMSO/PBS; applied 30 min before angiotensin II treatment; Sigma) and angiotensin II (0.1 μM in water; Sigma). Stimulation was performed 48 h after transfection and 24 h after starving. Harvest for RNA extraction or luciferase assay was performed 7 h after stimulation.

2.2. Tissue samples

Tissue samples from homozygous PARP-1 knockout mice (PARP-1 −/−) derived from the knockout line which was described previously [27]. Total RNA of these samples was isolated with the Trizol (Invitrogen, Karlsruhe, Germany) method. mRNA quantification by real-time PCR was performed based on six wild type and six knockout animals, each in technical triplicate. In brain tissue of these 6 + 6 mice, mRNA expression was evaluated based on three independent reverse transcriptions due to larger standard deviations.

The AT2R knockout mice were a kind gift of Michael Bader (Max Delbrück Center for Molecular Medicine (MDC), Berlin, Germany); real-time PCR data represent two animals in each group and technical triplicates.

2.3. Constructs

Serial deletion mutants of the human AT2R promoter were subcloned into the luciferase reporter vector pGL3-basic (Promega, Mannheim, Germany) using a common antisense primer located directly upstream of exon 2 (5′-gtaagagaaacagcagctaaataat) and following sense primers: 5′-atagcttgtaatagcacctaccata-3′ [-1316-construct], 5′-gagctgggtttgtaaagccaggtag-3′ [-1116-construct], 5′-tggttgtgtagtttattgccccccac-3′ [-916-construct], 5′-agccaacaaaaactgcgcaaagcaa-3′ [-716-construct], 5′-gaaaaaaggaagagaaaattctgta-3′ [-516-construct] and 5′-ctggcaagggttcataagtcagccc-3′ [-316-construct]. The [-1100] construct of human AT2R promoter was subcloned using an antisense primer located directly upstream of exon 1 (5′-aatagcaccagatctctggtttcct-3′) and the sense primer used for the [-1316] construct. The [-216] construct and [-148] construct based on the antisense primer located directly upstream of exon 2 (see above) and the sense primers 5′-acgtcccagcgtctgagagaacgag-3′ and 5′-taagtatgaacaatttatatataat-3′, respectively. The [-216-inverse] construct was subcloned as the [-216] construct but exchanging the restriction sites within the anchor primers.

Serial deletion mutants of the human ATIP1 promoter were subcloned into the luciferase reporter vector pGL3-basic (Promega) using a common antisense primer located directly upstream of the translational start site (5′-gtcttcggagcaggtggcgagattt-3′) and following sense primers: 5′-tattcgtggtttaatggaattagct-3′ [-1100-construct], 5′-gaaagaccactataagagaccac-3′ [-900-construct], 5′-cttttgacaatctgtcattaatatatt-3′ [-700-construct], 5′-acacagtttacttttcaggaaatcc-3′ [-500-construct], 5′-agcatgcacagtgtgatgtggt-3′ [-300-construct] and 5′-gtatttcttggccttgaagagtac-3′ [-100-construct].

A PCR product (5′-gggaggatggcggagtcttcggata-3′ (sense primer) and 5′-ttaccacagggaggtcttaaaattg-3′ (antisense primer)) comprising the complete open reading frame of human PARP-1 was subcloned into the expression vector pcDNA3.1-V5-His-TOPO (Invitrogen, Karlsruhe, Germany).

All constructs were confirmed by sequencing.

2.4. Quantitative RT-PCR

First-strand cDNA was synthesized by MLV-RT (Promega) using random hexamer primers (Promega). cDNA was quantified by real-time PCR using Power Mix (Applied Biosystems, Darmstadt, Germany). Data analysis was performed according to the $\Delta\Delta C_T$ method. Following primer pairs were used for real-time PCR: