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Research paper

Transcriptional regulation of heterogeneous nuclear ribonucleoprotein K gene expression

Liqing He [a, b], Xiaochang Xue [b], Zhengjun Wang [a], Entai Hou [a], Yong Liu [c], Mingyu Liang [c], Yingqi Zhang [b, **], Zhongmin Tian [a, *]

[a] Key Laboratory of Biomedical Information Engineering of Ministry of Education, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, China
[b] State Key Laboratory of Cancer Biology, Department of Biopharmaceutics, School of Pharmacy, The Fourth Military Medical University, Xi'an 710032, China
[c] Department of Physiology, Medical College of Wisconsin, Milwaukee, WI 53226, USA

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ABSTRACT

Heterogeneous nuclear ribonucleoprotein K (hnRNP K) is importantly involved in the regulation of development, DNA damage response, and several human diseases. The molecular mechanisms that control the expression of hnRNP K are largely unknown. In the present study, we investigated the detailed mechanism of the transcriptional regulation of human hnRNP K gene. Two activating and one repressive elements located in the proximal segment of the transcriptional initiation site were identified in hnRNP K gene. A 19 bp-region was responsible for the inhibitory activities of the repressor element. Twenty proteins were identified by DNA-affinity purification and mass spectrometry analyses as binding partners of the primary activating element in the hnRNP K promoter. Chromatin immunoprecipitation and EMSA analysis confirmed the binding of Sp1 with hnRNP K promoter. Sp1 enhanced the promoter activity, increased the expression of hnRNP K, and reduced the mRNA level of angiotensinogen, a gene known to be negatively regulated by hnRNP K. In summary, the current study characterized the promoter elements that regulate the transcription of human *hnRNP K* gene, identified 20 proteins that bind to the primary activating element of hnRNP K promoter, and demonstrated a functional effect of Sp1 on hnRNP K transcription.

1. Introduction

Heterogeneous nuclear ribonucleoprotein K (hnRNP K), which is present in both cytoplasmic and nuclear compartments [1,2], has been demonstrated to be involved in a number of fundamental biological processes. HnRNP K can regulate the transcription of several genes [3–9]. It was found to bind to the single-stranded C-rich sequence within the poly (Pu/Py) tract [8] or CT-rich element in repeat 3 region [9] to promote the transcription of vascular endothelial growth factor (VEGF) or low density lipoprotein receptor (LDLR). It can also bind to the mRNAs of many genes such as glucose-6-phosphate dehydrogenase (G6PD) [10] and renin [11] to suppress splicing or enhance stabilization. Bomsztyk K et al. found hnRNP K was bound to elongation factor 1α (EF-1α) [12], and a series of studies showed a role of hnRNP K in protein translation. The tyrosine and Ser302 of hnRNP K were phosphorylated by angiotensin II through Ang II-AT_1R-src-PKCδ pathway, which promoted the translation of VEGF mRNA [13,14]. Together with hnRNP E1and DEAD-box RNA helicase 6(DDX6), hnRNP K can also bind to the differentiation control element (DICE) in 3′ untranslated region (3′UTR) of 15-lipoxygenase mRNA to form a silencing complex, which could inhibit the translation of 15-lipoxygenase by control

Abbreviations: hnRNP K, heterogeneous nuclear ribonucleoprotein K; VEGF, vascular endothelial growth factor; LDLR, low density lipoprotein receptor; G6PD, glucose-6-phosphate dehydrogenase; AGT, angiotensinogen; ANG II, angiotensin II; AT_1R, Angiotensin Type-1 Receptor; DDX6, DEAD-box RNA helicase 6; SS-rat, salt sensitive rat; SS-13^{BN}, Consomic SS-Chr 13^{BN}/Mcwi rat; EF-1α, elongation factor 1α; SDS, sodium dodecyl sulfate; AP-1, activator protein 1; Sp1, specificity protein 1; KAP-1, KRAB-associated protein 1; ATM, ataxia telangiectasia mutated; ATR, ATM and Rad3-related; RFC1, Replication factor C subunit 1; ROS, reactive oxygen species; HCD, Higher Energy Collisional Dissociation; CNS, conserved non-coding sequence.

* Corresponding author. Tel.: +86 02982664955; fax: +86 02982668668.
** Corresponding author. Tel.: +86 83245677; fax: +86 84774775.
E-mail addresses: zhangyqh@fmmu.edu.cn (Y. Zhang), zmtian@mail.xjtu.edu.cn (Z. Tian).

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the assembly of 80s ribosomes [15,16]. Furthermore, hnRNP K contains more than seventy potential phosphorylation sites [17], suggesting it might be closely related to a variety of signal transduction pathways.

hnRNP K participates in the regulation of various physiological and pathological processes, including development [18–22], DNA damage response [23] and several human diseases [24–29]. It was reported recently that hnRNP K mRNA was gradually decreased in the rat central nervous system, but remained high in the peripheral nervous system throughout embryonic development [22]. Another study showed that the expression of hnRNP K was immediately induced by DNA damage [23]. Our previous study demonstrated that hnRNP K was up-regulated in the renal medulla of Consomic SS-Chr 13^{BN}/Mcwi rat (SS–13^{BN}) rats fed a 4% NaCl diet, but down-regulated in salt sensitive rat (SS) rats [30]. SS rats develop hypertension when fed high-salt diets. SS-13^{BN} rats are Consomic rats in which chromosome 13 in SS rats has been replaced by the corresponding chromosome from Brown Norway (BN) rats. Blood pressure salt-sensitivity was significantly reduced in SS-13^{BN} rats [30]. The down-regulation of hnRNP K in SS rats led to up-regulation of angiotensinogen (AGT) locally in the kidneys of SS rats [30,31], which might contribute to the development of salt-induced hypertension.

Given the important roles of hnRNP K in multiple physiological and pathological processes, it would be important to understand how the transcription of hnRNP K is regulated. However, little was known about the transcriptional mechanisms controlling the expression of hnRNP K. The purpose of current study is to identify and characterize the DNA sequence elements and transcriptional factors that regulate the transcription of *hnRNP K*.

2. Materials and methods

2.1. Cell culture

MCF-7, 293T and HK2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics (Invitrogen). Cells were maintained in a humidified 5% CO_2 atmosphere at 37 °C, the cell media were changed every 2 days, and the cells were subjected to passage every 3–4 days.

2.2. Plasmids construction

To construct luciferase reporter plasmids containing segments of *hnRNP K* proximal promoter (−992 to +217 bp, relative to the transcription start site +1), DNA fragments were amplified by PCR using Taq DNA polymerase (TaKaRa, Japan) with human genomic DNA as the template. The primer sequences are listed in Table 1. A *Kpn* I or an *Xho* I site was introduced into the sense and antisense primers respectively (underlined) and the length of each construct was shown in Fig. 1. The PCR conditions were as follows: 94 °C for 3 min; 30 cycles of 94 °C for 30sec, 60 °C for 30sec, and 72 °C for 90sec; and 72 °C for 10 min. PCR products were excised and purified from a 1.5% agarose gel, and cloned into the pMD 18T vector (TaKaRa, Japan). After identification by DNA sequencing, the target gene was recovered from the recombinant plasmid by digestion with *Kpn* I and *Xho* I and cloned into the pGL3-basic vector to construct the luciferase fusion plasmids. The fidelity of the promoter regions was further confirmed by the restriction enzyme digestion and DNA sequencing.

Table 1
Oligonucleotides used for plasmid construction.[a]

Oligo name	sequence(5′–3′)
K1-Forward	CGGGGTACCGCTTCGGGCACGAGTGTGGG
K1-Reverse	CCGCTCGAGGCCTTTCAGGGAGCCCCAACC
K2-Forward	CGGGGTACCGGGCGCCCCGGACCATTAC
K2-Reverse	CCGCTCGAGGGGACCGATGTTGCGCGAGG
K3-Forward	CGGGGTACCTGGGCGGAGGCTGGAAGGTT
K3-Reverse	CCGCTCGAGCGGTAAGTGCGGGCCGTCTG
K1–1-Forward	CGGGGTACCCTGTAAGGACAGGAACCGCCGC
K1–1-Reverse	CCGCTCGAGGCCTTTCAGGGAGCCCCAACC
K1–2-Forward	CGGGGTACCTCGGGGCTGGAAAACGCCC
K1–2-Reverse	CCGCTCGAGGCCTTTCAGGGAGCCCCAACC
K1–3-Forward	CGGGGTACCGGTTCGCCCCCTAGCCGCC
K1–3-Reverse	CCGCTCGAGGCCTTTCAGGGAGCCCCAACC
K1–4-Forward	CGGGGTACCGAGTGCGCGAACGAGAAAGGAGG
K1–4-Reverse	CCGCTCGAGGCCTTTCAGGGAGCCCCAACC
K1–5-Forward	CGGGGTACCGGGCGCTCCAGGCGACAGC
K1–5-Reverse	CCGCTCGAGGCCTTTCAGGGAGCCCCAACC
K1–6-Forward	CGGGGTACCACTGCAGACGCCATTATCCTCTGTTTC
K1–6-Reverse	CCGCTCGAGGCCTTTCAGGGAGCCCCAACC
K1–7-Forward	CGGGGTACCATCCTCTGTTTCTCTGCTGCACCG
K1–7-Reverse	CCGCTCGAGGCCTTTCAGGGAGCCCCAACC
K1–8-Forward	CGGGGTACCGCTGCACCGACCTCGACGTC
K1–8-Reverse	CCGCTCGAGGCCTTTCAGGGAGCCCCAACC
K1–9-Forward	CGGGGTACCCTTGCCTGTGTCCCACTTGTTCGC
K1–9-Reverse	CCGCTCGAGGCCTTTCAGGGAGCCCCAACC
K1–10-Forward	CGGGGTACCCTGTAAGGACAGGAACCGCCGC
K1–10-Reverse	CCGCTCGAGCTGCAGTGCTGTCGCCTGG
K1–11-Forward	CGGGGTACCCTGTAAGGACAGGAACCGCCGC
K1–11-Reverse	CCGCTCGAG ACTAGCTGGGGGGAGGGG
KN-sequence-F	CGGGCGCTCCAGGCGACAGCC
KN-sequence-R	TCGAGGCTGTCGCCTGGAGCGCCCGGTAC
K1–5sp*mut*-F	GGG *at* GGGGAAGGGGCGT
K1–5sp*mut*-R	CTCCCTGTGCGCGTGAACT
K2sp*mut*-F	GGGCTGTGGTCTGTGGGCTG
K2spmut-R	GGG AT GGGGTAAGGGGCTTCG
AGT-forward	CTCACTATGCCTCTGACCTGGA
AGT-reverse	CATGGTCAGGTGGATGGTCC
37 bp probe-F	GGAAAACGCCCCTTCCCCGCCCCCTCCCTGTGCGCGT
37 bp probe-R	ACGCGCACAGGGAGGGGGCGGGGAAGGGGCGTTTTCC
Mutant probe-F	GGAAAACGCCCCTTC*AAAAA*CCCCTCCCTGTGCGCGT
Mutant probe-R	ACGCGCACAGGGAGGGG*TTTTT*GAAGGGGCGTTTTCC

[a] A *Kpn* I or an *Xho* I site was introduced into the forward and reverse primers respectively(underlined).

2.3. Transient transfection

Sp1 overexpression plasmid was kindly provided by Dr. Guntram Suske at Philipps-University Marburg, Germany. The luciferase reporter plasmids were constructed as above. Cells were

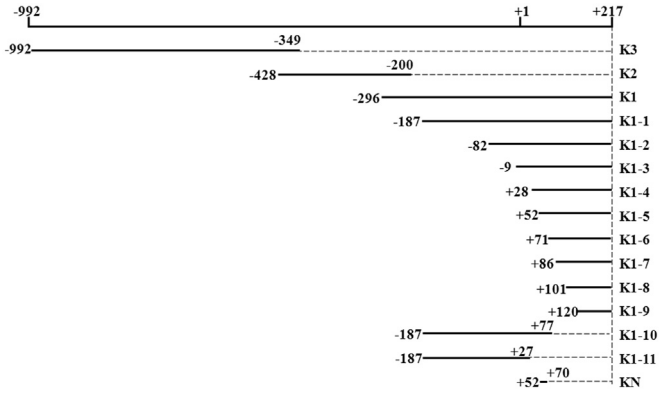

Fig. 1. All DNA segments analyzed in the current study. The numbers in the figure indicated the positions of start and end nucleotides, with +1/−1 indicating the transcription start site. The name of each construct was indicated on the right such as the K1–1 represents the sequence start from −187 bp to +217 bp.