The Journal of Applied Pharmacology, **15**, 78-82(2007)

Effects of Proto-oncogene Protein DEK on PCAF Localization

In-Seon LEE[1], Seok-Cheol LEE[1], Jaehwi LEE[2] and Sang-Beom SEO[1]*

[1]*Department of Life Science, College of Natural Sciences,*
[2]*Depatrment of Pharmaceuticals, College of Pharmacy, Chung-Ang University, Seoul 156-756, Korea*

(Received 22 May 2007; Accepted 14 June 2007)

Abstract – The proto-oncogene protein DEK is a nuclear binding phosphoprotein that has been associated with various human diseases including leukemia. Histone acetylation is an important post-translational modification which plays important role in transcriptional regulation. Auto-acetylation of histone acetyltransferase PCAF results in increment of its HAT activity and facilitation of its nuclear localization. In this study, we report that DEK inhibits PCAF auto-acetylation through direct interaction. The C-terminal acidic domains of DEK are responsible for the interaction with PCAF. Using confocal microscopy, we have shown that nuclear localization of PCAF is severely inhibited by DEK. Taken together, our results suggest that DEK may be involved in various cellular signal transduction pathways accommodated by PCAF through the regulation of PCAF auto-acetylation.

Keywords □ PCAF, DEK, Acetylation, Localization, Protein interaction, Nucleus

INTRODUCTION

In higher eukaryotes, the genomic DNA is compacted by association with nuclear proteins, including histones, and the resulting nucleoprotein complex is known as chromatin (Krude, 1995). The nucleosome comprises 146bp of DNA wrapped in nearly two turns around an octamer of histone proteins, made up of two copies of each of the core histones H2A, H2B, H3 and H4. Chromatin structure plays important regulatory roles in the control of gene expression in eukaryotes. The modulation of chromatin structure and transcription can be achieved though post-translational modifications of N-terminal histone tails such as acetylation, phosphorylation, methylation, ubiquitination, and sumoylation (Struhl, K, 1998). Histone modifications play major regulatory roles in man genetic events such as transcriptional activation and elongation, silencing and epigenetic cellular memory (Strahl *et al*., 2000; Turner, 2002). Activation of chromatin-assembled templates by histone acetyltransferases (HATs) such CBP/p300 and PCAF result in the disruption of the repressive structure to allow accessibility of basal factors.

Besides the general involvement of histone acetyltransferases, CBP/p300 and PCAF in transcriptional, recent studies suggest that different transcription factors show selective interaction with these coactivators and the exact role of the acetylation of transcription factors by these coactivators remains to be established (Song *et al*., 2002).

Among the HATs, PCAF is acetylated by itself and by p300 but not by CBP. The intramolecular acetylation targets five lysines (416-442 amino acids) at the central region of PCAF, which are in the nuclear localization signal (NLS). The acetylation of PCAF leads to an increment of its HAT activity, and facilitation of PCAF nuclear localization (Santos-Rosa *et al*., 2003).

The DEK protein was first identified in a fusion with the CAN nucleoporin protein, resulting from the t(6;9) chromosomal translocation occurring in acute myeloid leukemia (AML). This translocation results in the fusion of two genes, *dek* and *can*, and the expression of a leukemia-specific, chimeric *dek-can* mRNA and fusion protein (von Lindern *et al*., 1992). Like their normal counterpart DEK, DEK–CAN was localized exclusively to the nucleus. It was concluded that the relocation of the carboxyl-terminal portion of CAN from the nuclear envelope to the nucleoplasm may reinforce a nuclear function of CAN, implying that this relocation plays a role in leukemogenesis (Fornerod *et al*., 1995, 1996).

Recently, we have reported that the acidic domain containing protein DEK inhibits p300 and PCAF mediated histone acetylation and represses transcription (Ko *et al*., 2006). This suggests DEK might be involved in many cellular signal transduc-

*Corresponding author
Tel: +81-22-822-3059, Fax: +81-22-822-3059
E-mail: sangbs@cau.ac.kr

tion such as apoptosis through the regulation of enzymes responsible for the chromatin remodeling. In this study, we have extended DEK's role in this regulation by providing the evidences that inhibition of PCAF auto-acetylation by DEK. Furthermore, the inhibition is facilitated by direct interaction between DEK and PCAF via the acidic domain of DEK. Co-transfection of PCAF and DEK restrained PCAF localization to the cytoplasm which suggests regulation of PCAF activity by DEK.

MATERIALS AND METHODS

Plasmids and recombinant proteins

For bacterial and eukaryotic expression constructs of DEK and DEK: C200, the appropriate polymerase chain reaction (PCR)-amplified fragments were cloned into pGEX-4T1, pGEX-2TK and CMX-PL1 vectors, respectively. Sequences of all constructs surrounding the cloning sites were verified by automated sequencing. Recombinant proteins were expressed in BL 21 (DE3) *E. coli* cells (Novagen), purified using glutathione beads (Amersham-Pharmacia).

Cell culture

HeLa cells were maintained in Dulbecco's Modified Eagles Medium (DMEM, Gibco BRL) supplemented with 10% Fetal Bovine Serum (FBS, Gibco BRL) and penicillin-streptomycin (50 units/ml).

HAT assay

Purified total histones were obtained commercially (Roche Molecular Biochemicals). Nucleosomes were isolated form HeLa cells. HAT assays were performed in the presence of reaction buffer containing 50 mM Tris-HCl (pH 8.0), 10% glycerol, 0.1 mM EDTA, 0.1 mM PMSF, 10 mM sodium butyrate, and 0.15 μl of ^{14}[C]-acetyl coenzyme A (50 μCi/ml, 1000 pmol/ml, Amersham Biosciences). Baculovirus-expressed Flag-tagged PCAF were incubated with or without increasing amounts of purified wild type or mutant GST-DEK for 30min at 30°C. Reaction products were separated by 14% SDS-PAGE and analyzed by phosphorimager.

In vitro immunoprecipitation and interaction assay

For in vitro immunoprecipitation assays, baculovirus expressed Flag-PCAF were incubated with glutathione sepharose bound GST, GST-DEK, and GST-DEK :C200. Beads were extensively washed and analyzed in immunoblots using antibodies.

Immunoprecipitation and western analysis

HeLa cells were seeded in 60mm dishes with pCMX-DEK and Flag-tagged PCAF using lipofectamine 2000 (Invitrogen). 48 h after transfection, cells were harvested and processed according to the immunoprecipitation protocol. Western blotting was performed using standard procedure.

Immunostaining

HeLa cells were seeded in 4 well chamber slide and transfected with Flag-PCAF and pEGFP-DEK as indicated. After 48 h, cells were washed with phosphate-buffered saline (PBS) and fixed with methanol/acetone (1:1). After blocking with 1% Bovine Serum Albumin (BSA), cells were incubated with α-PCAF (Santa-cruz) antibodies, followed by incubation with Cy3-conjugated α-rabbit (Jackson ImmunoResearch Laboratories) and mounted with Gel/Mount (Biomeda). Images were examined utilizing confocal microscope (OLYMPUS FV300).

RESULTS

DEK inhibits PCAF auto-acetylation *in vitro*

Previous work has shown that PCAF is auto-acetylated (Santos-Rosa *et al*., 2003). Auto-acetylation of PCAF targets five lysines (416-442) at the center of PCAF. These target lysines are in the nuclear localization signal (NLS) domain of PCAF (Fig. 1A) and the auto-acetylation resulted in cytoplasmic localization of PCAF. Previously, we have shown that HAT activity of PCAF is inhibited by DEK *in vivo* and *in vitro* (Ko *et al*., 2006). To test whether DEK has same inhibitory activity towards auto-acetylation of PCAF, we performed HAT assay using baculovirus-expressed Flag-tagged PCAF and DEK purified as GST fusion proteins from *Escherichia coli.* Both recombinant proteins were incubated with ^{14}[C]-acetyl-CoA, in presence or absence of GST-DEKs, and auto-acetylation levels of PCAF were analyzed by phosphorimager. In the bottom panel, the bands in the coomassie gel (indicated with an asterisk) corresponds to a GST-DEKs. The auto-acetylation of PCAF was inhibited by addition of wild type DEK (Fig. 1B, lanes 3 and 4). Compare to the wild type DEK, DEK: C200 which lacks two acidic C-terminal domains and dose not have HAT inhibitory activity failed to inhibit the auto-acetylation of PCAF (Fig. 1B, lanes 5 and 6). Purified GST protein alone had no effect on the assay system (Fig. 1B, lane 2). These observations demonstrate that DEK inhibits auto-acetylation of PCAF

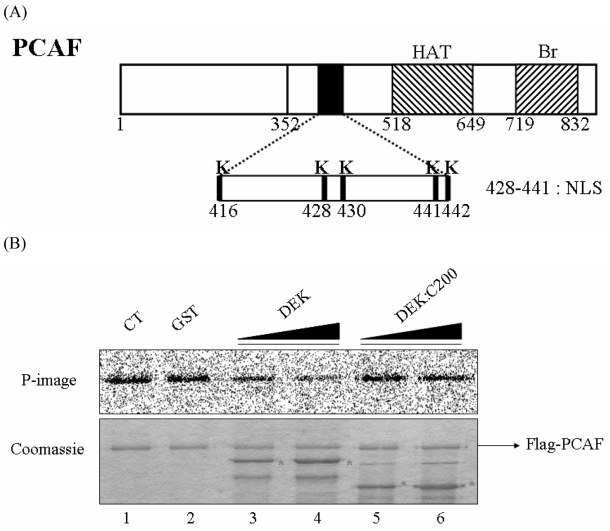

Fig. 1. DEK inhibits PCAF auto-acetylation *in vitro*. (A) Schematic representation of the full-length PCAF and NLS (352–658) region (acetylated lysines are indicated). HAT; HAT domain, Br; Bromo domain. (B) PCAF was incubated with wild-type and deletion mutant GST-DEK: C200 in HAT assay. Samples were separated by SDS-PAGE and analyzed by phosphorimager. Asterisks indicated purified GST-DEK proteins with molecular weights, 68 kDa (GST-DEK) and 51 kDa (GST-DEK: C200).

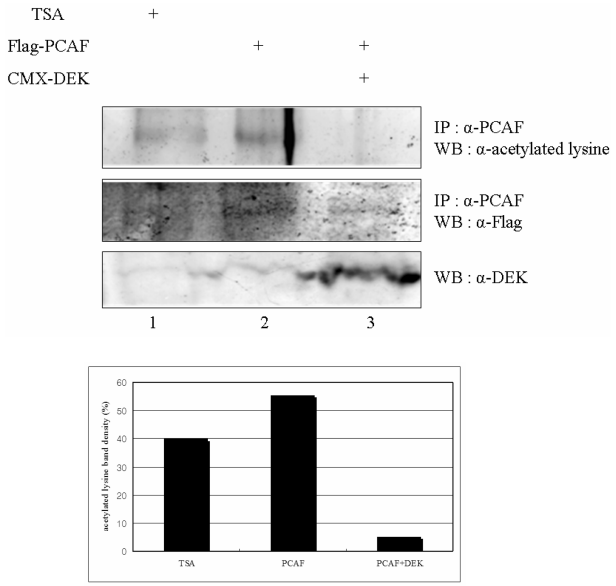

Fig. 2. DEK inhibits PCAF auto-acetylation *in vivo*. Flag-PCAF and pCMX-DEK plasmids were transfected into HeLa cells. Proteins were immunoprecipitated using antibodies against PCAF (upper and middle panels). The acetylation level of PCAF was analyzed by western blotting using the α-acetylated lysine antibodies (upper panel). The α-acetylated lysine band intensities were measured by densitometry and presented as bar graph in the lower panel.

possibly through the acidic domains of DEK.

DEK inhibits PCAF auto-acetylation *in vivo*

We then wanted to establish whether DEK inhibits auto-acetylation of PCAF *in vivo*. To determine the effect of DEK on auto-acetylation of PCAF, Flag-PCAF and DEK constructs were co-transfected in HeLa cell. PCAF proteins were immunoprecipitated using the α-PCAF antibody and their auto-acetylation level was determined by western blotting using α-acetylated lysine antibodies. Immunoblot result indicated that DEK inhibit auto-acetylation of PCAF (Fig. 2, lane 3). The results in Figure 2 indicate that endogenous PCAF is also acetylated by Trichostatin A (TSA) *in vivo* (Fig. 2, lane 1). This suggests that PCAF is auto-acetylated and it is inhibited by overexpressed DEK *in vivo*. It is possible that auto-acetylation site in NLS of PCAF could be masked by the acidic domain of DEK.

DEK binds to PCAF in the presence of acidic domain

To determine whether PCAF interacts with DEK directly, we performed *in vitro* binding assay using recombinant GST-DEK beads (Fig. 3). For that purpose, Flag-tagged PCAF was incu-

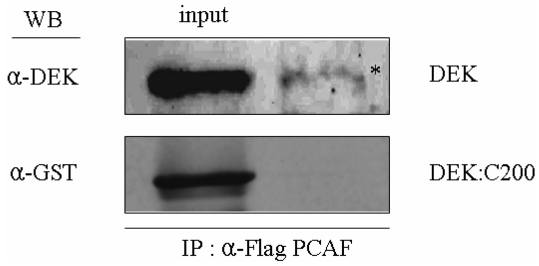

Fig. 3. DEK binds to PCAF in the presence of acidic domain. GST DEK and DEK: C200 were incubated with Flag-PCAF and immunoprecipitated with α-Flag antibodies. The bound proteins were detected by immunoblot analysis using α-DEK (DEK) or α-GST (DEK: C200) antibodies.

bated with wild type and DEK: C200 and immunoprecipitated with α-Flag antibodies. Immunoblot analysis using antibodies specific for DEK and GST were performed respectively (Fig. 3). The results showed wild type DEK interacted with PCAF. On the contrary, DEK: C200 did not bind to Flag-tagged PCAF. It indicated that the deletion of the C-terminal domain (amino acid 201-375) abrogates the interaction with PCAF *in vitro*. From this *in vitro* binding assay we have concluded that DEK directly interact with PCAF probably in its C-terminal acidic

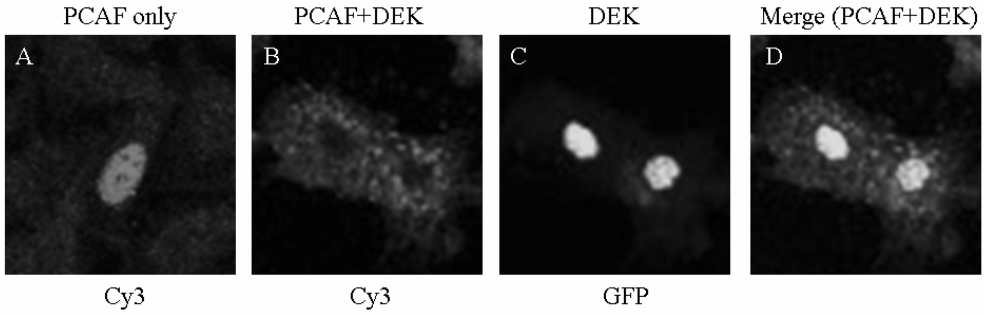

Fig. 4. DEK inhibits nuclear localization of PCAF. HeLa cells were co-transfected with GFP-tagged DEK and Flag-PCAF or Flag-PCAF only. Transfected cells were fixed and subjected to immunostaining with α-PCAF and analyzed by confocal microscopy.

domain dependent manner.

DEK inhibits PCAF nuclear localization

It has been proposed that auto-acetylation of PCAF might facilitate nuclear localization of PCAF in C2C12 cells before and after differentiation (Santos-Rosa *et al*., 2003). To further confirm whether DEK could influence the nuclear localization of PCAF, we co-transfected HeLa cells with GFP-DEK and Flag-PCAF and performed confocal microscopy. Clear nuclear localization was shown when Flag-PCAF was transfected alone (Fig. 4A). When Flag-PCAF and GFP-DEK were co-transfected, the localization of PCAF was exclusively in cytoplasm (Fig. 4B and C). These result indicated the inhibition of PCAF auto-acetylation in NLS sequence by DEK might affected nuclear localization of PCAF.

DISCUSSION

In this study, we presented here that PCAF is acetylated by itself and auto-acetylation is inhibited by DEK *in vitro*. Using in vitro HAT assay, we have shown that PCAF auto-acetylation is inhibited by the addition of DEK. Overexpression of DEK resulted in the decrease of PCAF acetylation level. Loss of inhibitory activity by deletion mutant DEK: C200 strongly suggests that the acidic domain of DEK might be responsible for the inhibition. Direct interaction between DEK and PCAF opens the possible secondary interaction by the other regulatory molecules such as HDACs and other co-repressors. As a strong histone H3 and H4 acetyltransferase, PCAF has been known to inhibits cell-cycle progression and regulates the mitogenic activity of the adenoviral oncoprotein E1A (Yang *et al*., 1996). It has been also suggested that the PCAF auto-acetylation could be involved in recruitment of transcription factors by affecting the PCAF-transcription factor interaction (Santos-Rosa *et al*., 2003). All together, our results opened the possibility of the regulation of various cellular PCAF activities by proto oncogene protein DEK. The further identification of exact inhibitory mechanism by DEK and a better understanding of its effects on nuclear localization of PCAF will help elucidate the effects of DEK on various cellular functions of PCAF.

ACKNOWLEDGEMENTS

This study was supported by a grant from Chung-Ang University.

REFERENCES

Fornerod, M., Boer, J., van Baal, S., Jaegle, M., von Lindern, M., Murti, K.G., Davis, D., Bonten, J., Buijs, A., and Grosveld, G. (1995). Relocation of the carboxyterminal part of CAN from the nuclear envelope to the nucleus as a result of leukemia-specific chromosome rearrangements. Oncogene 10: 1739-1748.

Fornerod, M., Boer, J., van Baal, S., Morreau, H., and Grosveld, G. (1996). Interaction of cellular proteins with the leukemia specific fusion proteins DEK-CAN and SET-CAN and their normal counterpart, the nucleoporin CAN. Oncogene 13: 1801-1808.

Ko, S.I., Lee, I.S., Kim, J.Y., Kim, S.M., Kim, D.W., Lee, K.S., Woo, K.M., Baek, J.H., Choo, J.K., Seo, S.B. (2006). Regulation of histone acetyltransferase activity of p300 and PCAF by proto-oncogene protein DEK, FEBS Letters 580, 3217-3222

Krude, T. (1995). Chromatin. Nucleosome assembly during DNA replication. Curr. Biol. 5: 1232-1234.

Santos-Rosa, H., Valls, E., Kouzarides, T., and Martínez-Balbás, M. (2003). Mechanisms of P/CAF auto-acetylation. Nucleic Acids Res. 31: 4285-4292.

Song, C.Z., Keller, K., Murata, K., Asano, H., and Stamatoyannopoulos, G. (2002). Functional interaction between coactivators CBP/p300, PCAF and transcription factor FKLF2. J. Biol. Chem. 277: 7029-7036.

Strahl, B. D., and Allis, C. D. (2000). The language of covalent

histone modifications. Nature 403: 41-45.
Struhl, K. (1998). Histone acetylation and transcriptional regulatory mechanisms. Genes Dev. 12: 599-606.
Turner, B.M. (2002). Cellular memory and the histone code. Cell 111: 285-291.
von Lindern, M., Fornerod, M., van Baal, S., Jaegle, M., de Wit, T., Buijs, A., and Grosveld, G. (1992). The translocation (6;9), associated with a specific subtype of acute myeloid leukemia, results in the fusion of two genes, dek and can, and the expression of a chimeric, leukemia-specific dek-can mRNA. Mol. Cell. Biol. 12: 1687-1697.
Yang XJ, Ogryzko VV, Nishikawa J, Howard BH, Nakatani Y. (1996) A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. Nature. 382(6589): 319-24.