Differential Intracellular Localization of Mitotic Centromere-associated Kinesin (MCAK) During Cell Cycle Progression in Human Jurkat T Cells

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Mitotic centromere-associated kinesin (MCAK), which is a member of the Kin I (internal motor domain) subfamily of kinesin-related proteins, is known to play a role in mitotic segregation of chromosome during M phase of the cell cycle. In the present study, we have produced a rat polyclonal antibody using human MCAK (HsMCAK) expressed in *E. coli* as the antigen. The antibody specifically recognized the HsMCAK protein (81 kDa), and could detect its nuclear localization in human Jurkat T cells and 293T cells by Western blot analysis. The specific stage of the cell cycle was obtained through blocking by either hydroxyl urea or nocodazole and subsequent releasing from each blocking for 2, 4, and 7 h. While the protein level of HsMCAK reached a maximum level in the S phase with slight decline in the G_2-M phase, the electrophoretic mobility shift from $p81^{MCAK}$ to $p84^{MCAK}$ began to be induced in the late S phase and reached a maximum level in the G_2/M phase, and then it disappeared as the cells enter into the G_1 phase. Immunocytochemical analysis revealed that HsMCAK protein localized to centrosome and nucleus at the interphase, whereas it appeared to localize to the spindle pole, centromere of the condensed mitotic DNA, spindle fiber, or midbody, depending on the specific stage of the M phase. These results demonstrate that a rat polyclonal antibody raised against recombinant HsMCAK expressed in *E. coli* specifically detects human MCAK, and indicate that the electrophoretic mobility shift from $p81^{MCAK}$ to $p84^{MCAK}$, which may be associated with its differential intracellular localization during the cell cycle, fluctuates with a maximum level of the shift at the G_2-M phase.

Key words – human MCAK, motor protein, rat polyclonal anti-HsMCAK, electrophoretic mobility shift, differential expression, cell cycle, Jurkat T cells

Kinesin-related proteins (KRPs) represent a fundamental component of the machinery for organelle movement in eukaryotic cells. KRPs contain a motor domain that moves cargo along microtubules by utilizing ATP, and thus are implicated in various intracellular transport events[16,22]. Unlike other kinesins that transport cargo, MCAK and the other members of the Kin I (internal motor domain) subfamily of kinesins are known to catalyze microtubule depolymerization rather than move along microtubule polymer [7,9]. This catalytic depolymerization by MCAK, which occurs at the end of mitotic spindle microtubule, appears to be an essential event for normal chromosome movement during M phase of the cell cycle. MCAK is detected throughout the cell but is dominantly located at the centromeres and kinetochores, and the spindle poles. Recently, it has been shown that in the *Xenopus* extracts, MCAK associates with Inner Centromere Kin I Stimulator (ICIS), by which ICIS is able to stimulate the microtubule depolymerizing activity of MCAK[17]. More recently, it has been shown that Aurora B kinase phosphorylates centromeric MCAK and regulates its localization as well as microtubule depolymerization activity[2,13]. In vitro, the phosphorylation of MCAK by Aurora B leads to the strong inhibition of MCAK ability to depolymerize the microtubules. At least 6 phosphorylation sites were found on the N-terminal region of MCAK, and the phosphorylation is thought to occur in living cells during the M phase[25]. Removal of Aurora B activity by treatment with small interfering (si) RNA of Aurora B[2], or with Hesperidin the Aurora B kinase inhibitor[13] blocked localization of MCAK to centromere, whereas immunodepletion of MCAK from *Xenopus* extracts failed to affect the accumulation of Aurora B at centromere[13]. In addition, Aurora B activity was not required for MCAK accumulation at the spindle poles[13]. These studies showing the functional role of MCAK and dependence of both subcellular localization and microtubule deploymerization activity of MCAK on its phosphorylation status regulated by Aurora B were mainly performed in *Xenopus* MCAK as well as mammalian hamster MCAK. Although the functional role of MCAK in

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during mitosis is thought to be conserved in metazoans, it needs to be clarified whether human MCAK is regulated in a same manner as it is in other organisms.

Recently we cloned human MCAK (HsMCAK) cDNA from human Jurkat T cells. As determined by DNA sequence analysis, the clone appeared to contain an open reading frame of 2169 bp, which encodes a 723-amino acids protein with a molecular mass of 81 kDa[10]. Northern blot analysis employing the cDNA as probe revealed that human MCAK-specific mRNA is expressed in tissues containing dividing cells, such as thymus, testis, small intestine, colon (mucosal lining), and placenta, and that the expression of HsMCAK specific mRNA may be induced in the S phase to support cellular proliferation. The functional role of human MCAK as a motor protein required for cellular proliferation remains largely unknown possibly due to unavailability of its specific antibody. Analysis of the amino acid sequence deduced from the nucleotide sequence of HsMCAK revealed that the kinesin motor domain signature sequence (GS)-(KRHSTQ)-(LIVMF)-x-(LIVMF)-(IVC)-D-L-(AH)-G-(SAN)-E[3-5,8,19] was in position 484~495 and the ATP/GTP-binding site motif A consisting of a (AG)-x(4)-G-K-(ST)[19,23] was located in position 346-353. While the overall sequence homology of human MCAK is 79.2%, 50.4%, 63.2%, and 33.7% identical to hamster MCAK, murine KIF-2, Xenopus XKCM1 and diatom DSK1, the sequence homology within motor domain shows 89.4%, 78.5%, 83.6%, and 45.0% identity to individual counterparts, respectively.

In the present study, we raised rat polyclonal antibody using recombinant human MCAK (HsMCAK) protein expressed in *Escherichia coli* system as the antigen, and sequentially the antibody were employed to investigate differential expression and subcellular localization of MCAK during the cell cycle of human Jurkat T cells were determined. The results show that the rat polyclonal antibody obtained after tertiary immunization specifically recognizes HsMCAK so that it can be applicable to Western blot analysis and Immunofluorescence microscopy. The results also indicate that the electrophoretic mobility shift of HsMCAK on SDS-PAGE, which may result from its phosphorylation, and subcellular localization of MCAK can be distinct during the cell cycle.

MATERIALS AND METHODS

Bacterial Strain and Vector plasmid

E. coli BL21(DE3)pLysS [*hsd*S gal (λcI*ts*857 *ind*1 *Sam*7 *nin*5 lacUV5-T7 *gene*1) *pLys*S] and the protein expression vector pET 3d were provided by Dr. Joel Shaper (The Johns Hopkins University, Baltimore, MD, USA). The recombinant pET 3d plasmid harboring a human MCAK cDNA fragment containing the open reading frame, which encodes 723 amino acid protein, in the *Nco*I/*Bam*HI site was designated pET 3d-HsMCAK.

Expression of human MCAK (HsMCAK) in *E. coli* and purification

To insert the cDNA fragment encoding the HsMCAK into the *Nco*I/*Bam*HI site of pET 3d vector, polymerase chain reaction was performed in the presence of HsMCAK cDNA as well as both *Nco*I-forward primer 5'-AATGGCCATGGACTCGTCGCTT-3' and *Bam*HI-reverse primer 5'-AGGATCCTCACCGAACTGAGTTC-3 (*Nco*I/*Bam*HI sites are underlined). PCR conditions were as follows: 30 cycles of denaturation at 94℃ for 1 min, annealing at 58℃ for 1.5 min, and extension at 72℃ for 2 min. The amplified cDNA fragment was cloned in the *Nco*I/*Bam*HI site of pET 3d vector (Aersham, Arlington Heights, USA), resulting in pET 3d-HsMCAK, in which the cDNA was placed under a strong lac promoter in sense orientation. The plasmids were then introduced into *E. coli* BL21(DE3) [*hsd*S gal (λcI*ts*857 *ind*1 *Sam*7 *nin*5 lacUV5-T7 *gene*1) *pLys*S, and transformants were selected on LB-containing ampicillin plates. The synthesis of HsMCAK protein in *E. coli* was induced by isopropyl β-D-thiogalactopyranoside (IPTG) as described previously [21]. To identify and localize the HsMCAK protein produced by the *E. coli* transformant, the bacterial culture was fractionated into three portions including total cell lysate, soluble protein fraction, and inclusion bodies (pellet) as described [14], and an equivalent amount of each fraction was electrophoresed on 11% SDS polyacrylamide gel. The presence of HsMCAK protein was determined by staining with Coomassie brilliant blue. The protein accumulated in the insoluble inclusion body fraction were purified from the gel by electroelusion after SDS-polyacrylamide gel electrophoresis.

Immunization of rat with the human MCAK

Four hundred microliters of the HsMCAK (100 μg) purified by electroelution was mixed with an equal volume of Complete Freund's Adjuvant, and were injected intramuscularly at the thigh muscle of two rear legs of a rat. For secondary and tertiary immunization, the protein mixed with Incomplete Freund's adjuvant was injected into the rat in

the same manner every 2 weeks. The bleeding was done 7 days after each immunization to test antibody titer.

Synchronization of cell cycle progression of Jurkat T cells by hydroxy urea or nocodazole

Continuously proliferating Jurkat T cells (4×10^5/ml) were cultured in RPMI 1640 medium with 1 mM of hydroxy urea for 20 h to block the cell cycle progression at late G_1 phase[1], whereas Jurkat T cells were cultured in the presence of 0.1 μg/ml of nocadazole for 20 h to arrest at the G_2/M boundary[6]. To release the cells from the arrest points and to continue the cell cycle progression, the cells treated with hydroxy urea or nocadazole were layered over FBS, centrifuged, washed three times with 1X HBSS containing 2% FBS, and then incubated in RPMI 1640 medium up to 7 h. The culture medium used for human Jurkat T cells was RPMI 1640 (Bethesda Research Laboratories) containing 10% FBS (Upstate Biotechnology Inc., Lake placid, NY), 20 mM HEPES (pH 7.0), 5×10^{-5} M β-MeOH, and 100 μg/ml gentamycin.

Flow cytometric analysis

Cell cycle progression of resting T cells following polyclonal activation was analyzed by Flow cytometry as described elsewhere[11]. Approximately 1×10^6 T cells were suspended in 100 μl of PBS, and 200 μl of 95% ethanol were added while vortexing. The cells were incubated at 4℃ for 1 h, washed with PBS, and resuspended with 12.5 μg of RNase in 250 μl of 1.12% sodium citrate buffer (pH 8.45). Incubation was continued at 37℃ for 30 min before staining of the cellular DNA with 250 μl of propidium iodide (50 μg/ml) for 30 min at room temperature. The stained cells were analyzed on a FACScan flow cytometer for relative DNA content, based on increased red fluorescence.

Cell lysate, Subcellular Fractionation, Protein Quantitation, and Western blot analysis

The cells were suspended in lysis buffer (137 mM NaCl, 15 mM EGTA, 1 mM Na_3VO_4, 15 mM $MgCl_2$, 25 mM MOPS, 1 mM phenylmethylsulfonyl fluoride, 2.5 μg/ml E-64, 0.1% Triton X-100, pH 7.2), and disrupted by sonication and extracted at 4℃ for 30 min as described elsewhere[18]. After centrifugation at 14000 rpm for 20 min, the supernatant was obtained as cell lysate. For preparation of cytoplasmic and nuclear fractions, the cells were treated as described previously[15]. Protein quantitation was performed using Micro BCA kit (PIERCE, Rockford, IL). Total 15 μg of cell lysates were subjected to electrophoresis on an 4~12% NuPAGE gradient gel and electrotransferred to Immobilon-P membrane. The membrane was allowed to react with individual primary antibodies and then with horse radish peroxidase conjugated with Donkey anti-rabbit IgG antibody. Detection of each protein was visualized by ECL Western blotting detection system according to the manufacturer's instructions.

Immunofluorescence Microscopy

Human Jurkat T cells were adhered onto glass cover slips pretreated with 2% aminopropyltriethoxysilane for 30 min in a humidified chamber as previously described[11,12], and fixed with cold methanol for 3 min. The cells were rinsed four times with cold PBS containing 0.5% Triton X-100, and blocked with 10% goat serum for 30 min. The cells were then incubated with monoclonal anti-α-tubulin (1:500) or rat polyclonal anti-MCAK (1:600) overnight at 4 °C. For detection, the cells were treated with FITC-conjugated goat anti-mouse immunoglobulin (1:100) or TRITC-conjugated goat anti-rat immunoglobulin (1:250) for 1 h at room temperature. Image was visualized and photographed using Nikon Microphot Fluorescence Microscope or Leica Confocal Laser Scanning Microscope.

RESULTS AND DISCUSSION

Construction of Recombinant Plasmid pET 3d-HsMCAK and Expression in *E. coli*

To produce human MCAK in *E. coli*, the cDNA corresponding to the entire open reading frame of 2,169 bp, which encodes a 723-bp amino acid protein with molecular mass of 81 kDa, was inserted into the *Nco*I/*Bam*HI site of an expression vector pET 3d and designated pET 3d-HsMCAK. MCAK cDNA fragment required to construct pET 3d-HsMCAK was obtained by PCR. When PCR was done with HsMCAK cDNA in the presence of both *Nco*I-forward primer and *Bam*HI-reverse primer, 2,169-bp PCR product was amplified (Fig. 1A). The PCR product was purified by extraction with buffer-saturated phenol buffer and precipitation with 2.5 volume of cold ethanol. Subsequently, it was further purified on an Elutip-D column (Schleicher & Schuell, Keene, NH, USA). The purified PCR product (60 ng) was treated with *Nco*I/*Bam*HI and then ligated with 0.1 μg of pET 3d vector that was linearized by the same restriction enzymes.

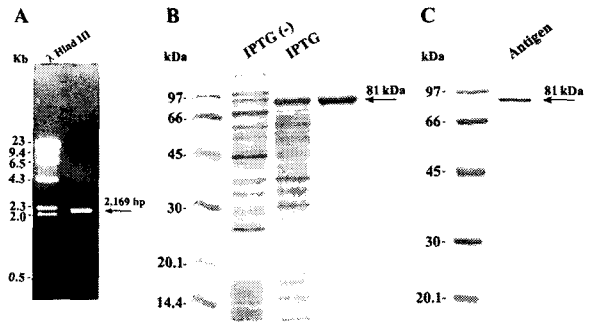


Fig. 1. Electrophoresis of HsMCAK cDNA fragment after amplification by PCR (A), and identification of the HsMCAK protein in insoluble fraction after expression in *E. coil* (B), and confirmation of the purified HsMCAK protein by electroelution along with SDS-PAGE (C).

After the ligation mixture was used for transformation of *E. coli* BL21(DE3)pLysS, the transformant containing the pET 3d-HsMCAK was obtained. From the transformant, the pET 3d-HsMCAK was purified and the nucleotide sequence of inserted coding region for the open reading frame (723 aa) of HsMCAK was confirmed (data not shown). The *E. coli* transformant containing pET 3d-HsMCAK was designated *E. coli* pET-HsMCAK.

For identification of the HsMCAK protein produced in *E. coli* pET-HsMCAK, the strain was cultured with shaking in LB media containing ampicillin (50 μg/ml) and chloramphenicol (20 μg/ml) at 37℃, and when growth O.D at 600 nm reached at 0.4~0.5, 0.3 mM IPTG was added and cultivation was continued for additional 4 h. The *E. coli* expression system employing pET vectors was developed not only to overexpress target DNAs under control of a T7 promotor and T7 RNA polymerase but also to regulate the expression of target DNAs by placing a T7 RNA polymerase gene under control of *lac*UV5 promotor, which is inducible by IPTG[21]. In addition, the expression system was constructed to express target DNAs from its own translation start and not as a fusion protein. As shown in Fig. 1B, the HsMCAK protein was not induced without IPTG, whereas it induced in the presence of 0.3 mM IPTG, and molecular mass was 81 kDa as expected from the reading frame on the DNA sequence. When the bacterial culture was sonicated and then fractionated into the soluble portion and the insoluble inclusion body portion, the HsMCAK protein was mainly localized in the portion of the inclusion body (Fig. 1B). To purify of the HsMCAK protein, the inclusion body fractions were harvested from the culture of *E. coli* pET-HsMCAK. Since the inclusion body fraction appeared to contain several additional proteins upon SDS-PAGE, which were distinct in molecular mass from 81 kDa of the HsMCAK protein, the HsMCAK protein was further purified by electro-elution from the gel. As shown in Fig. 1C, the purified protein of the HsMCAK was confirmed on 11% SDS-PAGE. These results demonstrate that the expression of the HsMCAK protein is successfully induced by IPTG in *E. coli* pET-HsMCAK, and the protein is mainly localized in the inclusion body.

Production of Rat Polyclonal Antibody Using the HsMCAK as the Antigen

In order to produce a rat polyclonal antibody against HsMCAK protein, 200 μg of the purified recombinant HsMCAK protein mixed with Adjuvant was injected intramuscularly for each primary, secondary, and tertiary immunization. To evaluate the antibody titer by Western blot analysis, bleeding from the rat was done 7 days after each immunization. Since the antiserum obtained 10 days after tertiary immunization in 2000-fold dilution was able to detect specifically the HsMCAK protein with molecular mass of 81 kDa in the cell lysates of human leukemia Jurkat , human kidney cell line 293T and human leukemia K562, the animal was sacrificed and the antiserum was recovered (Fig. 2A). Since MCAK has been reported as a nuclear protein in Chinese hamster ovary (CHO) cells[24], we have also examined whether the 81-kDa protein recognized by rat anti-MCAK antibody is detectable in the nuclear fraction or in cytoplasmic fraction of Jurkat T cells. As shown in Fig. 2B, the 81-kDa protein detected in both Jurkat T and 293T cells by rat anti-MCAK was mainly localized in the nuclear fraction. These results indicate that a rat polyclonal anti-human MCAK antibody was successfully produced in rat immunized with the recombinant human MCAK expressed in *E. coli*.

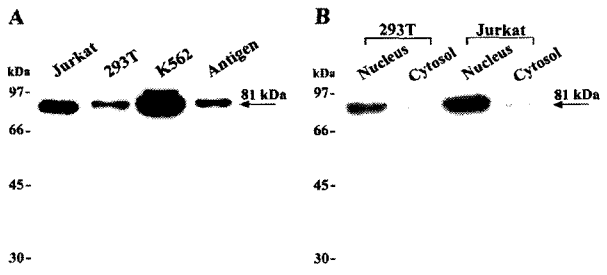
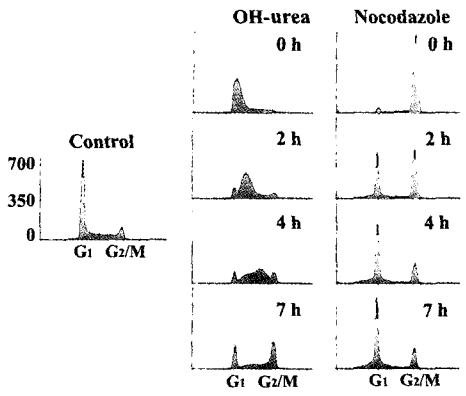


Fig. 2. Western blot analysis of HsMCAK (81 kDa) expressed in Jurkat, 293T, and K562 cells, and the antigen employed to raise rat polyclonal antibody (A), and nuclear localization of HsMCAK in 293T and Jurkat cells.

Dectection of Electrophoretic Mobility Change and Intracellular Localization of HsMCAK Protein During Cell cycle Progression of Jurkat T Cells

In *Xenopus*, aurora B kinase has been shown to phosphorylate centromeric MCAK and regulate its localization to centromere as well as its microtubule depolymerization activity, whereas aurora B kinase activity is not required for its localization to spindle poles[2,13]. Although the functional role of MCAK in mitotic segregation of chromosome during the cell cycle is expected to be conserved throughout eukaryotic cells, it still remains unclear whether the relations between MCAK phosphorylation by aurora B kinase and subcellular localization of MCAK observed in *Xenopus* consistently occurs in human cells. Furthermore, it is unknown whether the phosphorylation of human MCAK fluctuates during the cell cycle progression. Since the hyperphosphorylated forms of several cellular proteins have been shown to migrate slowly on SDS-PAGE as compared to their dephosphorylated or hypophosphorylated counterparts, it seems likely that the phosphotylation status of HsMCAK during the cell cycle can be detectable by Western blot analysis. To confirm that HsMCAk is differentially phosphorylated during the cell cycle, the alteration in the electrophoretic mobility of HsMCAK during the cell cycle of Jurkat T cells was investigated by Western blot analysis using rat polyclonal anti-HsMCAK. As shown in Fig. 3A and 3B, the continuously growing Jurkat T cells (Control) were composed of 57% of the G_1 cells, 27% of the S phase cells, and 14% of the G_2/M phase cells. Treatment of Jurkat T cells with hydroxy urea or nocadazole for 20 h efficiently blocked the cells at the G_1/S transition point or at the G_2/M boundary; approximately 81% of the cells remained at the G_1 and 84% of the cells at the G_2/M phase, respectively. Since both hydroxy urea[1] and nocodazole[6] are known as reversible blocking agents of the cell cycle progression, reversal of the blocking was easily accomplished by washing the cells and resuspending in the reagent-free medium at 37℃. The blocked cells could synchronously continue the cell cycle progression at least until 7 h after releasing them from the blocking. To obtain Jurkat cells at the specific stages of the cell cycle, the cells were released from the blocking point for 2, 4 or 7 h. Subsequently, the cells were processed to prepare cell lysate. Western blot analysis revealed that the expression of MCAK protein reached a maximum level in the S phase with slight decline in the G_2-phase, and showed a minimum level as the cells

A



B

	Percentage (%)		
	G_1	S	G_2/M
Control	**56.50**	**26.70**	**13.60**
OH-urea blocking	**80.58**	**11.02**	**4.92**
2 h release	**12.18**	**77.22**	**7.64**
4 h release	**10.10**	**71.56**	**15.05**
7 h release	**22.35**	**29.45**	**42.50**
Nocodazole blocking	**4.18**	**5.89**	**84.15**
2 h release	**33.01**	**9.51**	**47.29**
4 h release	**51.19**	**10.61**	**22.77**
7 h release	**59.35**	**10.71**	**19.80**

Fig. 3. Flow cytometric analysis (A) and quantification (B) of the cell cycle distribution of continuously growing Jurkat T cells. Jurkat T cells were cultured in RPMI 1640 medium with 1 mM of hydroxy urea for 20 h to arrest at late G_1/S boundary or with 0.1 μg/ml of nocadazole to arrest at the G_2/M boundary, and the cells released from the arrest points for the indicated time periods. Jurkat T cells in individual conditions were harvested and stained with propidium iodide, and an equal number of cells (10^4) were analyzed by flow cytometry.

moved into the G_1 phase (Fig. 4A). Under these conditions, the hyperphosphorylation of HsMCAK, which results in electrophoretic mobility shift from $p81^{HsMCAK}$ to $p84^{HsMCAK}$, began to be detectable when the cells exit from the S phase and reached a maximum level at the G_2/M phase. However, the slow-migrating $p84^{HsMCAK}$ declined as the cells exit from G_2/M phase and then disappeared at the G_1 phase. Under these conditions, the protein level of two cell cycle regulatory proteins cdc2 and cyclin B, which are known to form a complex and play an important role in the M phase, was also determined (Fig. 4B and 4C). The level of cdc2 protein was relatively constant throughout the cell cycle progression, whereas cyclin B1 level was fluctuated and reached a maximum in the G_2/M phase, as described previously[20]. The level of α-tubulin, which is known to

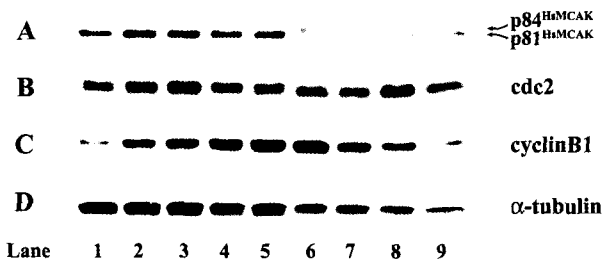


Fig. 4. Western analysis of the changes in the protein level of HsMCAK (A), cdc2 (B), cyclin B1 (C), and α-tubulin (D) in Jurkat T cells blocked in late G_1 phase by treatment of hydroxy urea or blocked at the G_2/M boundary by nocadazole, and released from each blocking point for various time periods. Fifty micrograms of cell lysate extracted from Jurkat T cells untreated (lane 1), cells blocked with hydroxy urea (lane 2) and released from the blocking for 2 h (lane 3), 4 h (lane 4) and 7 h (lane 5) or cells blocked with nocadazole (lane 6) and released from the blocking for 2 h (lane 7), 4 h (lane 8) and 7 h (lane 9) were analyzed as described in Materials and Methods.

form microtubule, remained at a maximum in the S phase, but it decreased slightly as Jurkat cells traverse G_2/M and enter into G_1 phase (Fig. 4D). These results demonstrate that rat anti-HsMCAK specifically recognize both dephosphorylated and hyperphosphorylated HsMCAK. These results also indicate that the phosphorylation status of HsMCAK, which can be easily detectable by Western blot analysis employing rat anti-HsMCAK, fluctuates during the cell cycle progression with a maximum level of phosphorylation at the G_2/M phase. To examine subcellular localization of HsMCAK in continuously growing Jurkat T cells, the cells were adhered onto glass cover slips pretreated with 2% aminopropyltriethoxysilane, and then the localization of HsMCAK was analyzed by immunocytochemistry using mouse monoclonal anti-α-tubulin and rat polyclonal anti-HsMCAK. HsMCAK appeared to dominantly localize to centrosome as well as nucleus at the interphase, whereas it localized to spindle pole and centromere of the condensed mitotic chromosome at the prometaphase and metaphase, and to midbody at the telophase of the cell cycle (Fig. 5). These results demonstrate that rat polyclonal anti-HsMCAK raised against recombinant HsMCAK overexpressed in *E. coli* specifically recognize HsMCAK and can be applicable to Western blot analysis and immunocytochemical analysis of HsMCAK. These results also indicate that the cell cycle-dependent subcellular localization of MCAK protein previously observed in *Xenopus* and hamster models is well conserved in human system.

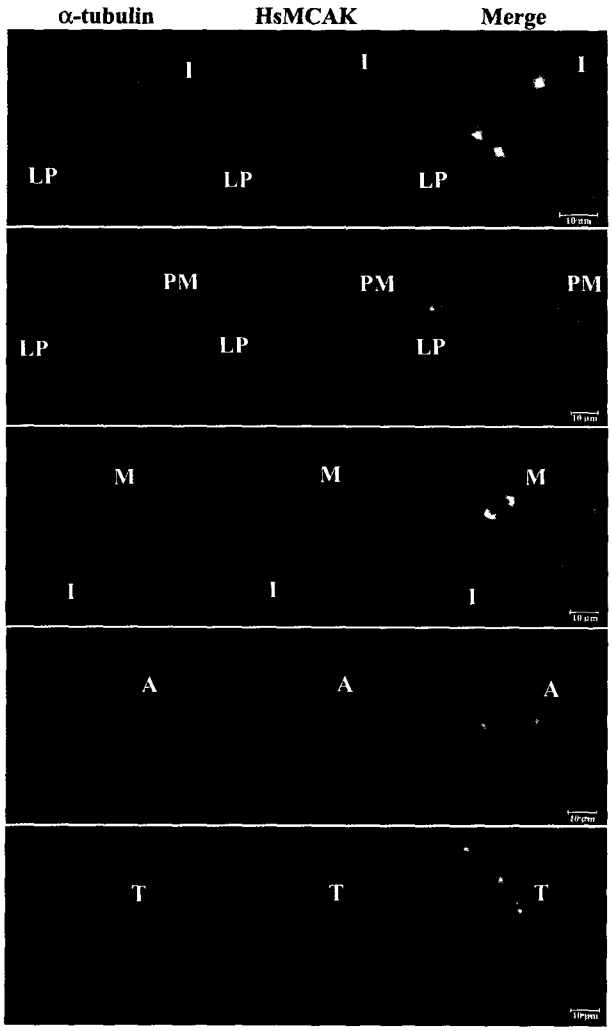


Fig. 5. Immunocytochemical analysis of subcellular localization of HsMCAK during cell cycle of Jurkat T cells. Human Jurkat T cells adhered onto glass cover slips pretreated with 2% aminopropyltriethoxysilane were incubated with monoclonal anti-α-tubulin or rat polyclonal anti-MCAK. The cells were then treated with FITC-conjugated goat anti-mouse immunoglobulin (1:100) or TRITC-conjugated goat anti-rat immunoglobulin (1:250) as described in Materials and Methods. Symbols: I, interphase; LP, late prophase; PM, prometaphase; M, metaphase; A, anaphase; T, telophase.

In summary, these results demonstrate that rat polyclonal anti-MCAK raised against recombinant HsMCAK expressed *E. coli* is specific enough to detect HsMCAK on Western blot or in methanol-fixed Jurkat T cells. In addition, these results first demonstrate that the electrophoretic mobility shift from $p81^{HsMCAK}$ to $p84^{HsMCAK}$ was first detectable when the cells enter into G_2/M phase, and reached a maximum level at the G_2-M phase, whereas the slow - migrating $p84^{HsMCAK}$ declined as the cells exit from G_2/M phase and

then disappeared at the G_1 phase. HsMCAK appeared to primarily localize to centrosome, spindle pole, centromere of the condensed mitotic chromosome, or midbody during cell cycle progression.

Acknowledgements

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초록 : 인체 Jurkat T 세포에 있어서 세포주기에 따른 MCAK 단백질의 세포 내 위치변화

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인체 MCAK 단백질을 *Escherichia. coli*에서 재조합 단백질로 발현하였다. 이를 SDS-PAGE 후 electroelution으로 정제하고 항원으로 사용하여 rat에서 다클론성 항체생성을 유도한 결과, 생성된 항체는 Western blot analysis에 의해 인체 MCAK 단백질 (81 kDa)을 특이적으로 인식할 수 있었으며, Jurkat T cells과 293T cells에 있어서 MCAK 단백질의 대부분이 핵 내에 위치함을 확인할 수 있었다. 세포주기에 따른 MCAK 단백질의 발현양의 변화를 조사하기 위해, Jurkat T cells을 Hydroxy urea 또는 Nocodazole의 처리로 G_1/S boundary 그리고 G_2/M boundary에 blocking하고 이로부터 release 시키는 시간을 달리하여 다양한 세포주기상에 위치한 Jurkat T cells을 확보하였다. 각각의 Jurkat T cells로부터 cell lysate를 얻어서 Western blot analysis를 시도한 결과, MCAK 발현양은 S phase에서 가장 높았으며 MCAK의 SDS-PAGE상의 mobility가 81 kDa에서 84 kDa로 shift 됨을 확인하였다. MCAK의 전기영동상의 mobility shift에 의한 slow moving $p84^{HsMCAK}$는 S phase 후반부터 나타나기 시작하며 G_2/M phase에 최대였고 G_1 phase에서는 확인되지 않았다. 이는 세포주기에 따라 MCAK의 단백질의 인산화 양상이 달라짐을 시사한다. 생성된 항체를 이용한 Immunocytochemical analysis의 결과, 인체 MCAK 단백질은 세포주기의 interphase에서는 주로 중심체와 핵에 존재하며, M phase의 각 단계에 따라서 spindle pole, centromere, spindle fiber 또는 midbody에 존재함을 확인하였다. 이러한 연구 결과는 *E. coli*에서 발현된 재조합 HsMCAK 단백질을 항원으로 하여 rat에서 생산한 다클론성 항체가 HsMCAK 단백질을 특이적으로 인식할 수 있음과 또한 HsMCAK 단백질의 인산화를 나타내는 SDS-PAGE상의 mobility-shift가 G_2-M phase에 최대에 도달하는 양상으로 세포주기에 따라 변동됨을 나타내며, HsMCAK의 인산화와 HsMCAK의 세포 내 위치간의 관련성을 시사한다. 아울러 이러한 연구결과는 hamster 및 Xenopus 등에서 주로 연구되고 있는 MCAK의 세포주기상의 주요기능이 인체세포에도 적용될 수 있음을 시사한다.