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MS-KIF18A, a Kinesin Is Associated With Estrogen Receptor

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Abstract The study of MS-KIF18A kinesin protein is focused on its cellular distribution and association with a cargo protein. Indirect immunofluorescence (IF) analyzed the intracellular distribution of endogenous MS-KIF18A and the transfected enhanced green fluorescence protein (eGFP)-MS-KIF18A in osteogenic cells. In both cases, the proteins were localized at the plasma membrane, cytosol, and nucleus. Bioinformatics analysis suggested interactions between MS-KIF18A and estrogen receptor (ERα) which were further elucidated by immunoprecipitation (IP). We identified interaction between endogenous MS-KIF18A with 66 and 46 kDa isoforms of ERα in MBA-15 cells. Moreover, MS-KIF18A and 66 kDa ERα complex has been demonstrated between ectopically expressed proteins in COS-7 cells. We have shown that anti-MS-KIF18A antibody immunoprecipitated the ERα and pERK in cells challenged with 17β-estrogen (17β-E2). The hormone activation induced mitogen-activated protein kinases (MAPK) pathway and increased p-ERK. The activation was interfered when cells were pre-treated with either ICI-182,780 or MAPK inhibitor PD98059 prior the challenge with 17β-E2 that resulted in a decrease in association between MS-KIF18A and p-ERK1/2. The obtained results suggest a role for the proteins in a non-genomic response of MBA-15 cells challenged with 17β-E2. This study presents a novel interaction between MS-KIF18A and ER that may have important physiological and pharmacological implications for estrogen action in various cells. J. Cell. Biochem. 100: 693–702, 2007.

Key words: kinesin; estrogen receptor; protein interaction; imaging; signaling

Kinesins are microtubule-dependent motor proteins that function as cellular "cars," which transport organelles, protein complexes, including signaling modules and transcription factors and mRNA [Vale, 2003]. Structurally, kinesins consist of three functional parts: a motor domain that reversibly binds microtubules and converts chemical energy into motion, a coiled coil domain which possesses protein–protein interactions, and a tail which interacts with cargo [Vale and Milligan, 2000]. In particular, kinesins interface with intracellular protein trafficking and signal transduction. However, knowledge of the cargo carried along microtubules by kinesin motors is still missing. We previously described MS-KIF18A, a member of the kinesin super family. MS-KIF18A was analyzed by bioinformatics; biochemistry and imaging localizes the protein intracellular distribution and interactions with microtubules [Luboshits and Benayahu, 2005]. In the present work, we elaborate on the interaction of kinesin with a nuclear receptor (NR). Specifically, this study sheds light on the association between newly identified MS-KIF18A kinesin and the estrogen receptor (ERα) in the estrogen signaling pathway.

The estrogen effect on cellular metabolism is mediated by two receptors (ERs): ERα [Walter et al., 1985] and ERβ [Kuiper et al., 1996], which belong to the steroid NR superfamily. The receptor consists of several domains: the A/B domain at the N-terminal encodes the ligand-independent activation function domain (AF1). AF-1 is responsible for protein–protein interactions and transcriptional activation of target genes. The DNA-binding domain (DBD) mediates the receptor binding to promoters of estrogen-regulated genes. Region D is a flexible hinge region between DNA and the ligand-binding domains (LBD) [Ruff et al., 2000;

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Nilsson et al., 2001]. The C-terminal consists of the AF-2 domain, which is involved in interactions with transcriptional co-activators via NR boxes, LXXLL-motifs [Mak et al., 1999; Klinge, 2000]. ERα and ERβ have been detected in various cells, including skeletal cells: osteoblasts [Colvard et al., 1989; Shamay et al., 1996; Benayahu, 1997], osteocytes [Braidman et al., 2000], and osteoclasts [Oreffo et al., 1999]. ERα is expressed by two splice forms; the 66 kDa and 46 kDa, which lacks the AF-1 domain [Flouriot et al., 2000; Denger et al., 2001]. The ER binds its ligand in the cytoplasm and is then translocated to the nucleus. The receptor binds to estrogen response element (ERE) [Nilsson et al., 2001] or, through transcription factors such as AP-1 [Kushner et al., 2000] and SP1 [Batistuzzo de Medeiros et al., 1997] on promoters of responsive genes. The nuclear signaling of estrogen occurs within 30–60 min after hormonal treatment. An alternative rapid (seconds to minutes) pathway is activation of mitogen-activated protein kinases (MAPK) of proteins such as p38 and ERK1/2 [Ho and Liao, 2002], an increase in ion concentration [Morley et al., 1992; Massas et al., 1998] or inositol 1,4,5-trisphosphate (IP3) [Lieberherr et al., 1993] which are mediators of non-genomic actions of estrogen.

The ER is localized to different cellular compartments, including cell membrane, cytoplasm, and nucleus is known to shuttle dynamically in the cell, thereby dictating the physiological role of estrogen.

The study aimed at elaborating on the interaction between MS-KIF18A and ERα. Kinesins have a role in trafficking of proteins, thus play a role in signaling. We have demonstrated for the first time the interaction between MS-KIF18A (a motor protein) and a NR in the pathway of estrogen signaling.

MATERIALS AND METHODS

In Vitro Culture

Ex vivo primary cultured human mesenchymal marrow cells (MSC) [Shur et al., 2002] and MBA-15, a mouse marrow stromal derived cell line [Benayahu et al., 1989] were cultured in growth medium containing Dulbecco's modified essential medium (DMEM) (Gibco) with the addition of 10% heat-inactivated fetal calf serum (FCS, Biological Industries, Bet-Haemek, Israel). COS-7 cells were grown in DMEM medium supplemented with 5% FCS.

Estrogen Signaling, ERK1/2 Pathway

MBA-15 cells were maintained in medium supplemented with 3% charcoal-stripped FCS for 48 h before the experiment. On the day of the experiment the cells were pre-treated with 25 μM MAPK inhibitor, PD98059 (Calbiochem), ICI 182,780 10^{-6} M (Tocris) for 45 min and 10^{-8} M 17β-E2 (Sigma) was added for 5 min. Cell lysates were used for immunoprecipitation (IP) and analyzed by Western blot.

Immunoprecipitation Western Blot, SDS–PADE Gel

The cells were washed with ice-cold phosphate-buffered saline (PBS) and collected with PBS in presence of protease inhibitors (1 mM phenylmethylsulfonyl fluoride (PMSF), 1-chloro-3-tosylamido-4-phenyl-2-butanone, TPCK, 10 μg/ml; aprotinin, 10 μg/ml (Sigma) and phosphatase inhibitors cocktails I and II (Sigma)). Pellets of cells were lysed in buffer of 50 mM Tris pH 7.5, 150 mM NaCl, 1% NP-40, protease and phosphatase inhibitors, for 20 min at 4°C, and centrifuged at 16,000*g* for 5 min. Supernatants were collected and used for IP or analyzed as whole cell lysates. IP was performed with antibodies to MS-KIF18A [Luboshits and Benayahu, 2005] or anti-His-tag (Santa Cruz Biotechnologies) and protein A-sepharose beads (Sigma). Immunoprecipitated proteins were resolved on 8% SDS–PAGE gel and transferred to nitrocellulose membrane. Membranes were blocked with 5% BSA in TBST (50 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Tween-20, Sigma) and incubated with anti-MS-KIF18A, anti-ER antibodies (Upstate Biotechnologies) or (SRA-1010, Stressgen, Canada), anti-ERK1/2, pERK1/2 (Sigma) or anti-His-tag (Santa Cruz Biotechnologies). For detection we used secondary antibody conjugated to biotin-extravidine-peroxidase and chemiluminescent substrate (Pierce).

Plasmids

We cloned MS-KIF18A into two tagged vectors. (**A1**) Full-length MS-KIF18A was amplified using specific primers (5-CG-GGA-TCC-TCA-ACA-ATG-TCT-GTC-ACT-GAG; 3-C CG-CTC-GAG-GAT-CAA-CTT-CAT-TTT-GCT-TGG) and cloned into pcDNA 3.1-His-C vector (Invitrogen). The resulting pcDNA3.1-His-C-MS-KIF18A vector was confirmed by sequen-

cing. (**A2**) Enhanced green fluorescence protein (eGFP)-MS-KIF18A was produced based on plasmid pcDNA3.1-His-C-MS-KIF18A. The insert was restricted with KpnI and XbaI (New England Biolab) and cloned to C-terminus of EGFP-C3 vector (Clontech) (**A3**) pCMV-IL-2R was kindly provided by Dr. G. Hager, NCI, NIH, USA. (**A4**) pSG5h-ERα66 was kindly provided by Dr. F. Gannon, EMBL, Heidelberg, Germany.

Transfection Experiments

Expression constructs pcDNA3.1-His-C-MS-KIF18A and pSG5hER66 were transiently transfected into COS-7 cells using PolyFect reagent (Qiagen). Cells were transfected with 1.5 μg DNA for each vector in co-transfection experiments and 2.5 μg DNA in a single transfection. After 16 h, cells were harvested for IP and subsequent Western blot analysis. For microscope analysis, MBA-15 cells were grown on cover slips and transiently transfected with 1 μg DNA for EGFP-MS-KIF18A, 3 μg for pCMV-IL-2R using Fugen6 reagent (Roche). On the following day, cells were fixed and processed for immunofluorescence (IF) staining.

Microscopy Analysis

(**A**) Confocal immunofluorescence microscopy; transfected and non-transfected MBA-15 cells were fixed in 4% paraformaldehyde in PBS for 20 min, permeabilized with 0.1% Triton in PBS for 5 min on ice. Cells were stained with primary antibodies anti-MS-KIF18A [Luboshits and Benayahu, 2005], anti-IL-2R (Upstate Biotechnologies), anti-GM130 (Transduction Laboratories), anti-KDEL receptor, anti-Lamin B (Santa Cruz) or anti-caveolin-1 (Transduction Laboratories) for 1 h at room temperature (RT). Secondary antibodies conjugated to either fluorescein isothiocyanate (FITC), or Texas-Red (Jackson Immuno Research Laboratories) were reacted on slides with appropriate primary antibody for 1 h at RT. Nuclei were stained with 4′,6′-diamidino-2-phenylindole hydrochloride (DAPI, Molecular Probes). Slides were mounted using Prolong anti-fade mounting media (Molecular Probes). Images were acquired on a confocal microscope (Zeiss 510, Germany). (**B**) Transmission electron microscopy (TEM) of cells fixed in 4% paraformaldehyde in PBS, pH 7.4 for 1 h at RT followed with post-fixed with 1% osmium tetra-oxide in PBS, pH 7.4 for 2 h dehydrated in ethanol and embedded in araldite. Ultra-thin sections were cut with a diamond knife in LKB ultra microtome and sections were mounted onto formavar-coated grids. The grids were stained with uranyl acetate and lead citrate, and the sections were examined with a transmission electron microscope (Jeol, JEM 100CII).

RESULTS

The cloning of MS-KIF18A, a kinesin protein and its cellular localization, was described earlier [Luboshits and Benayahu, 2005]. This study demonstrates the intracellular distribution of MS-KIF18A and EGFP-MS-KIF18A in transiently transfected MBA-15 cells. The IF performed with the anti-MS-KIF18A observed on confocal microscopy (Fig. 1A–C) revealed the distribution of EGFP-MS-KIF18A in the cytosol and cell nucleus (Fig. 1A). Anti-MS-KIF18A antibody staining demonstrated the same distribution (Fig. 1B) with an overlap between the fluorescent signals of the immunostaining and the EGFP signal (Fig. 1C) which confirmed that the expression of the transfected and endogenous proteins are the same subcellular localization. MS-KIF18A is observed in the nucleus, dispersed throughout the cytoplasm, and localized at the plasma ruffles. MBA-15 cells stained with anti-Lamin B antibody, a nuclear membrane protein, did not co-localize with EGFP-MS-KIF18A (Fig. 1D–F). In addition, the localization of EGFP-MS-KIF18A was compared with ectopically expressed interleukin-2 receptor (IL-2R). EGFP-MS-KIF18A that localizes at the plasma membrane (Fig. 1G) was partially co-localized with transfected IL-2R stained with anti-IL-2R (Fig. 1H,I) suggesting that MS-KIF18A is associated at plasma membrane structures. These results led us to analyze the caveolae membrane system in MBA-15 cells by TEM which were observed as cell-surface invaginations (Fig. 2A; arrows). Further IF analysis for caveolin protein using anti-caveolin-1 identified its localization in patches scattered along the plasma membrane and at cytoplasmic areas (Fig. 2C,G). Double staining with anti-MS-KIF18A and anti-caveolin-1 antibodies identified a partial co-distribution of these proteins in patches scattered along the plasma membrane (Fig. 2, arrows) and through the cytoplasm, (yellow is overlay of the two proteins, Fig. 2D,E,H),

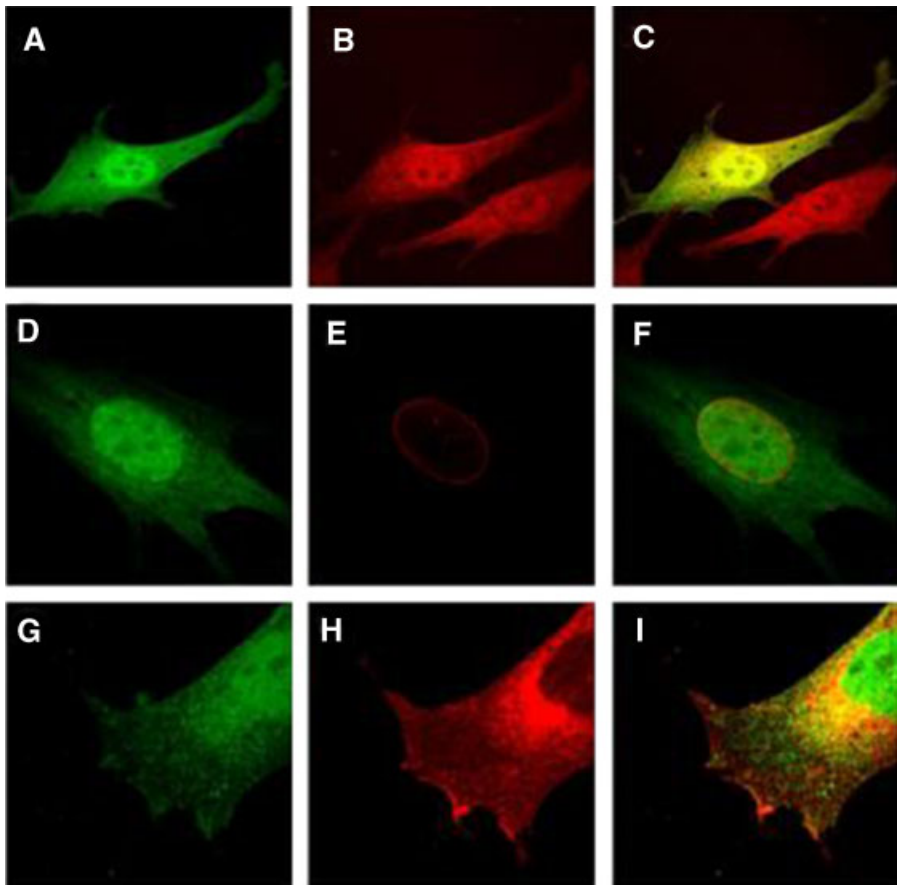

Fig. 1. Intracellular localization of EGFP-MS-KIF18A transfected in MBA-15 cell was analyzed by IF. Transiently transfected cells with EGFP-MS-KIF18A (**A**, **D**, **G**) were stained with anti-MSKIF18A antibody (Texas Red) (**B**), anti Lamin B (FITC) (**E**), or co-transfected with pCMV-IL-2R and stained with anti-IL-2R (**H**), Overlay images (**C**, **F**, **I**). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

indicating that the MS-KIF18A is associated with the caveolae membrane system. We analyzed also the distribution of MS-KIF18A with GM130 and Co-KDEL. IF of GM130, a Golgi matrix protein, function in the late steps of ER-Golgi traffic, did not co-localize with MS-KIF18A (Fig. 3A–C) or when cells were stained with anti-MS-KIF18A and anti-KDEL receptor, an endoplasmic reticulum receptor (Fig. 3D–F).

Next, we explored the potential cargo protein associates with MS-KIF18A. The N-terminus of MS-KIF18A is a motor domain that is highly conserved among kinesins. The C-terminus includes the stalk and the cargo-binding domain that are divergent between kinesins and responsible for cargo binding. Bioinformatics analysis of MS-KIF18A identified three NR boxes, the LxxLL motifs, and two I/LXXI/V (FXXFF) like motifs at the coiled coil and cargo-binding domains of the protein. These motifs mediate protein–protein interaction between transcriptional co-activators and between co-repressors and NRs. The cargo domain of MS-KIF18A contains a region homologous to the boundary region between the hinge and LBD of ERα (28.6% of identity). The coiled coil region of MS-KIF18A is homologous to the C-terminal of ERα-LBD (31% identity). The existence of these motifs in the sequence of MS-KIF18A led us to analyze possible interaction of the protein with ERα. To apply this approach we used anti-MS-KIF18A antibody to validate the association of the kinesin with ERα that were ectopically expressed in COS-7 cells (Fig. 4A) and between endogenous proteins in MBA-15 cells (Fig. 4B). COS-7 null cells were co-transfected with His-MS-KIF18A and ERα 66 kDa, and their co-expression enables to demonstrate an interaction of ERα 66 kDa with MS-KIF18A in transfected cells. IP experiment performed with

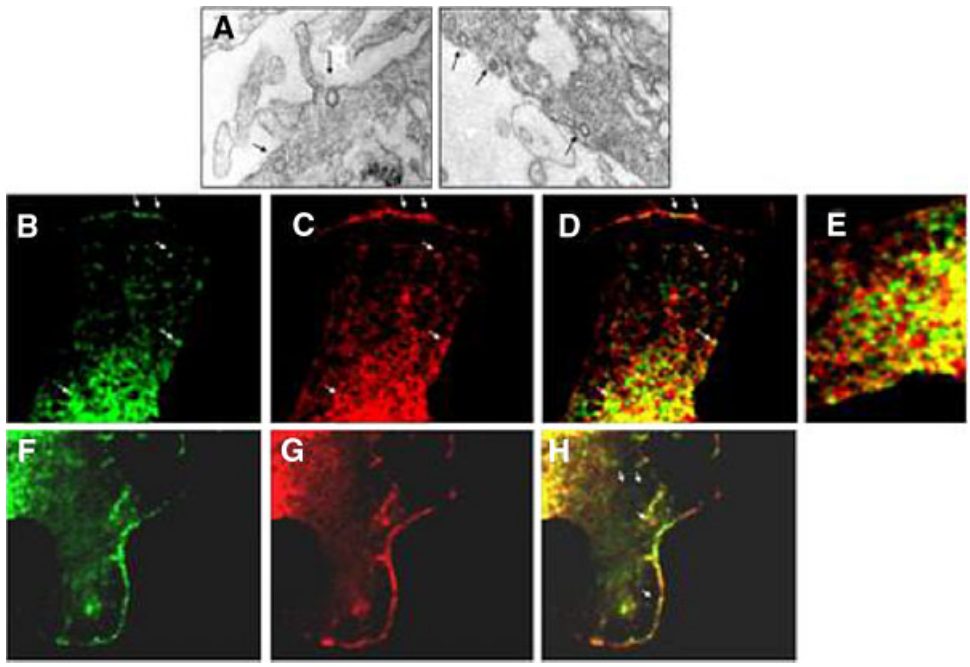

Fig. 2. Transmission electron microscopy micrograph of MBA-15 cells demonstrates the caveolae structures (**A**) at the plasma membrane region (arrows). Co-localization of MS-KIF18A and caveolin-1 demonstrated by IF (**B–H**). MBA-15 cells were stained with anti-MS-KIF18A (FITC) (B, F), anti-caveolin-1 (Texas Red) (C, G), overlay image (D, E, H). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

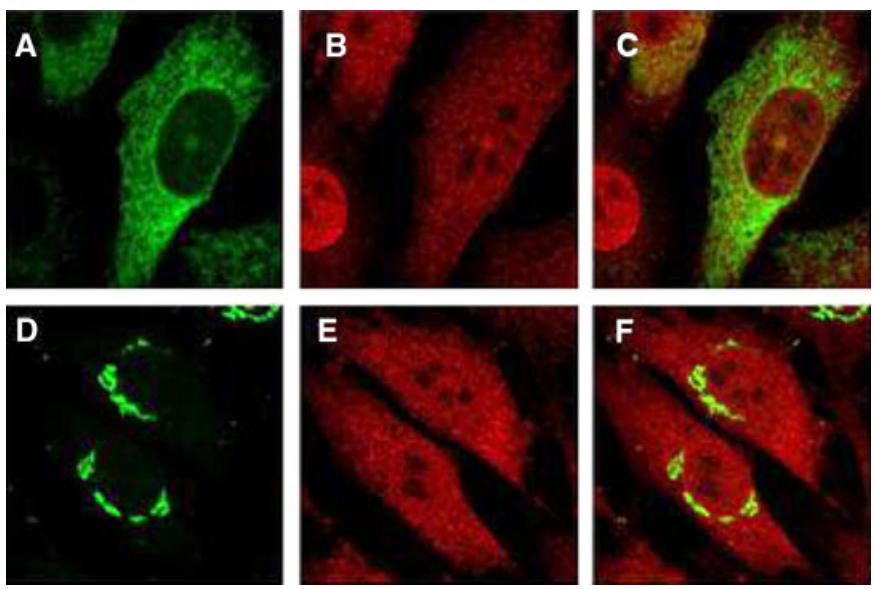

Fig. 3. IF used to visualize the MS-KIF18A in cellular compartment related to Golgi complex and endoplasmic reticulum. MBA-15 cells were stained with anti-MS-KIF18A (Texas Red) (**B**, **E**), with anti-KDEL receptor (FITC) for endoplasmic reticulum (**A**) or with anti-GM130, Golgi matrix protein (FITC) (**D**). Overlay images (**C**, **F**). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

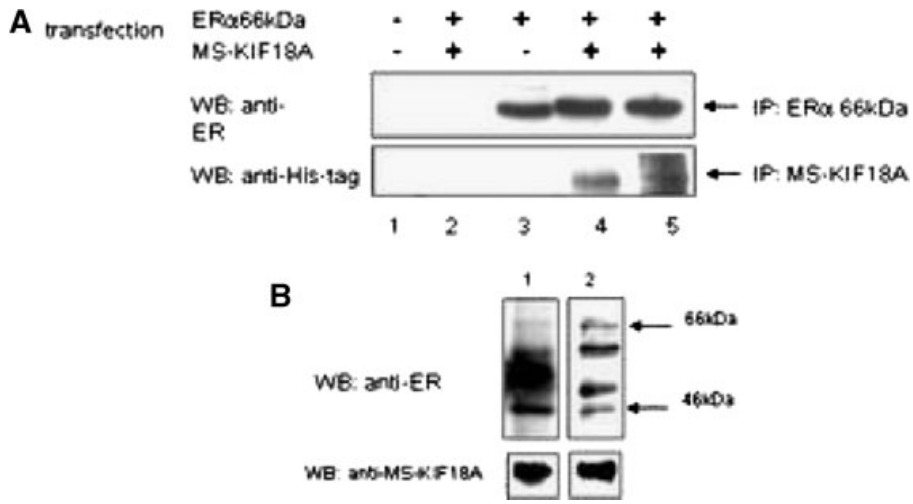

Fig. 4. Analysis of MS-KIF18A and ERα association in (**A**) transfected COS-7 cells (**B**) endogenous expressed proteins in MBA-15 cells. A: His-MS-KIF18A and ERα 66 KD were co-transfected into COS-7 cells analyzed for the protein interactions. 1: Whole cell lysates from non-transfected and (3) whole lysate from ERα66 transfected cells. Immunoprecipitation with beads only (2), with anti-His (4) anti-MS-KIF18A (5) Western blot performed with anti-ER and anti-His-Tag antibodies. B: Endogenous expressed MS-KIF18A and estrogen receptor were analyzed on immunoprecipitates from MBA-15 cells using anti-MS-KIF18A and Western blot analysis with anti-ERα (1) SRA-1010, (2) AER311 or anti-MS-KIF18A. Western blot with anti-ERα antibodies revealed association of various splice forms that were immunoprecipitated with MS-KIF18A (arrows).

the anti-MS-KIF18A or anti-His antibodies (Fig. 4A) and Western blot with anti-ERα antibody demonstrated pull down of ERα 66 kDa by the anti-MS-KIF18A and anti-His antibodies (Fig. 4A). The results confirmed the interactions between transfected His-MS-KIF18A and ERα 66 kDa. The association between endogenous proteins was analyzed in marrow stroma MBA-15 cells (Fig. 4B). Cell lysates were immunoprecipitated with anti-MS-KIF18A, and were applied for Western blot with two anti-ERα antibodies (SRA-1010 and AER311) which recognized different epitopes of the ERα (Fig. 4B). The IP result with pull down and revealed the association between MS-KIF18A and 66-kDa and 46-kDa isoforms of ERα in the MBA-15 cells. IP analysis with a non-relevant antibody (control) did not pull down the ERα (data not shown). Thus, the co-transfection experiments of both proteins in COS-7 cells (Fig. 4A) confirm the results obtained on the endogenous proteins in MBA-15 cells (Fig. 4B), that MS-KIF18A and ERα form protein complex that was pulled down by anti-MS-KIF18A antibody.

The estrogen effect on cell metabolism is mediated via genomic (longer) or non-genomic (short) responses. The non-genomic response is quick and characterized by the activation of the MAPK signaling pathways. MBA-15 cells were challenged with 17βE2 to measure its effect on activation of ERK1/2. Some cells were pre-treated with either ICI 182,780 or PD98059 and then exposed to 17βE2. Western blot of whole cell lysates demonstrated an increase of pERK1/2 following 17βE2 treatment that was inhibited in presence of ICI 182,780 or PD98059 (Fig. 5A). The data demonstrated that in MBA-15 cells, the non-genomic response of estrogen is associated with activation of ERK1/2. Furthermore, we analyzed the association of MS-KIF18A with ERα (Fig. 5B) and pERK1/2 (Fig. 5D) in response to 17βE2. MBA-15 cells were treated with estrogen, ICI 182,780 or PD98059 under the same experimental procedure as described above, were subjected to IP with anti-MS-KIF18A antibody and analyzed by Western blot (Fig. 5B–D). An association between MS-KIF18A and ERα is shown in Figures 4 and 5B and results in Figure 5D demonstrate that pERK1/2 is also accounted in MS-KIF18A–ERα complex. An increase in activated ERK was observed after estrogen treatment (Fig. 5D-4), while pre-treatment with PD98059 (Fig. 5D-2) or ICI 182,780 (Fig. 5D-3) reduced ERK activation (Fig. 5E). Collectively, the data demonstrate that in MBA-15 cells, estrogen response is associated with ERK1/2 activation and an association was demonstrated between MS-KIF18A and ERα.

The current study describes interaction between MS-KIF18A, ERα, and pERK in cells

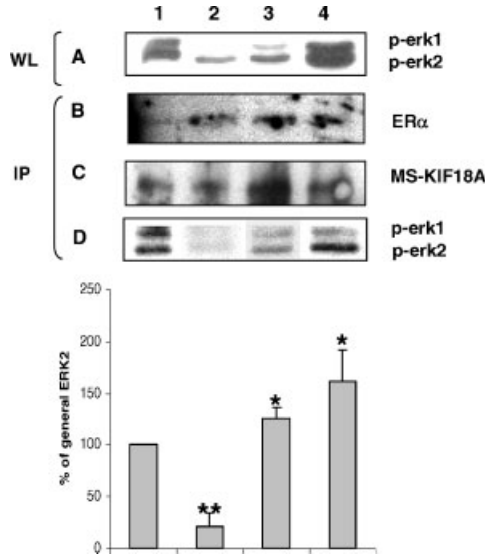

Fig. 5. MBA-15 cells untreated control (**Lane 1**), or cells treated with 17βE2 for 5 min; cells were pre-treated with PD98059 (PD) (**Lane 2**), ICI 182,780 (ICI) (**Lane 3**); cells treated with 17βE2 only (**Lane 4**). Western blot measured for p-ERK1/2 expression on whole cell lystes (**A**). Immunoprecipitation with anti-MS-KIF18A was then analyzed with either ERα (**B**), MS-KIF18A (**C**), or pERK (**D**). pERK levels were quantified and presented by bar graph (**E**) summarized three independent experiments; control compared to ICI + E ($*P < 0.05$), control compared to PD98059 ($**P < 0.005$).

challenged with estrogen. The co-localization of MS-KIF18A with caveoline-1 was demonstrated in osteogenic stromal cells. The pattern of intracellular localization of MS-KIF18A suggests that this protein can navigate between cellular compartments, with different modifications that affect the protein's metabolic status.

DISCUSSION

The MS-KIF18A is a new member of the kinesin superfamily, described in an earlier study [Luboshits and Benayahu, 2005] by bioinformatics, imaging, and biochemistry analysis. Kinesins are motor proteins that move along microtubules, using the energy derived from ATP hydrolysis to transport organelles and protein complexes within the cells [Goldstein, 2001; Gunawardena and Goldstein, 2004]. The kinesins that adapt to different cargo linkers governing protein–protein interactions play a role in protein trafficking and signal transduction. The current study describes the association between MS-KIF18A and ERα and enlightens the localization of these proteins in osteogenic stromal cells. MS-KIF18A localizes to different cellular compartments, including the nucleus and the cytosol. Herein, we have shown that MS-KIF18A localized at the plasma membrane, is associated with caveolin-1. Caveolae are lipid rafts known to participate in membrane signaling in a variety of cells, including osteoblasts [Solomon et al., 2000]. Caveolin-1 is the structural protein of the caveolae that decorates the membranes of cells and of caveosome bodies, "cavicles." Cavicles are transported from caveolae to the centrosomal region on microtubules; their trafficking suggested the role of kinesins in this movement [Pelkmans et al., 2001; Mundy et al., 2002]. Caveolae were demonstrated by TEM and confocal imaging in MBA-15 cells. Anti-caveolin-1 has shown a scattered pattern at the cells' membranes and at the peri-nuclear region, representing the caveosome. IF demonstrated an association between MS-KIF18A and caveolin-1 at the plasma membrane and in caveosome. MS-KIF18A also localized at the nucleus relies on the NLS motifs identified in the cargo-binding domain and conserved sumoylation motifs [Luboshits and Benayahu, 2005]. These motifs affect the dynamics of protein trafficking between nucleus and cytosol. The intracellular pattern of MS-KIF18A suggests that this protein can navigate between cellular compartments upon different modifications that affect the protein's metabolic status. MS-KIF18A is not associated with the nuclear membrane as no co-localization was observed with a nuclear lamina protein, Lamin B. Other kinesins, such as KIF5C and KIF5B, formed a complex with the nuclear pore protein, RanBP2 [Mavlyutov et al., 2002].

Motor proteins' unique function is their interaction with specific cargo. However, to date, only a few cargo molecules have been identified. To analyze for potential cargo for MS-KIF18A, we employed bioinformatics followed by biochemical experiments. The bioinformatics predictions suggested the interaction between MS-KIF18A and ERα relies on homology between the MS-KIF18A at the coiled coil and cargo domains with the ERα hinge and LBD. Moreover, the existence of LXXLL and I/LxxI/V (FXXFF) like motifs in the sequence of MS-KIF18A is recognized in mediating the interaction with the NR, co-activator, and co-repressor [Martini and Katzenellenbogen, 2003]. Such an association was confirmed using biochemical analyses on MBA-15 and COS-7 cell lysates.

CO-IP confirmed this association of endogenous proteins in MBA-15 cells and co-transfected proteins in COS-7 null cells. The proven interaction between MS-KIF18A and ERα is unequivocal. In an earlier study, the association of ER with cytoskeleton proteins was suggested; however, no candidate has been proposed yet to underlie these observations [Zafar and Thampan, 1995].

The complex between MS-KIF18A and ERα along with the knowledge on the nature of these proteins at different cellular compartments indicates a role for kinesin in ERα trafficking within the cell. Today, more then 50 kinesins are recognized; however, specific cargos have been identified for only a few proteins. For example, KIF13A binds cargo vesicles of AP-1 and mannnose-6-phosphate receptor (M6PR) [Nakagawa et al., 2000]. KIF17 interacts with mLin-10 (Mint1/X11) in neuronal cells, forming a large complex with other proteins [Setou et al., 2000]. KIF17b is associated with ACT, expressed at high levels exclusively in testis. KIF17b has been detected in two compartments, nucleus and cytoplasm, and has been demonstrated to direct the transcriptional activator in the cell [Macho et al., 2002].

ERα is localized at the caveolar fractions of the plasma membrane [Kim et al., 1999; Chambliss and Shaul, 2002]. Caveolin-1 was shown to facilitate the translocation of ERα, thus, the endogenous caveolin-1 and ERα are associated both at the cytosol and plasma membrane [Razandi et al., 2003]. Several members of the MAPK protein including ERK1/2 have been localized to caveolae [Li and Nord, 2004; Yang et al., 2004]. The membranous ER transduces the estrogen signal that is associated with a rapid activation of the MAPKs. This phenomenon was shown in variety of tissues and cells [Ho and Liao, 2002; Bulayeva et al., 2004; Chaban et al., 2004; Razandi et al., 2004].

Estrogen stimulates intracellular signaling via activation of phospholipase C, adenylate cyclase, resulting in increased production of inositol lipid and cAMP, respectively and an increase in intracellular Ca^{++} and activation of MAPKs: ERKs, JNKs, and p38 and phosphatidylinositol-3-kinase (PI3-kinase) [Collins and Webb, 1999; Moggs and Orphanides, 2001]. ERK activation in variety of cells, including bone cells, has an anti-apoptotic effect that is abolished when cells were pre-treated with an estrogen antagonist. In this study, we have shown an association between MS-KIF18A and p-ERK proteins, which play a role in the non-genomic activation induced by estrogen. Such activation was abolished in the presence of an estrogen antagonist, ICI 182,780 or ERK inhibitor, PD98059. Our results demonstrated the complex relationship between MS-KIF18A and ERα and suggest that MS-KIF18A is involved in non-genomic activation of the MAPK pathway by estrogen in osteogenic cells.

MS-KIF18A shares sequence homology with two other kinesins: Klp67A (*Drosophila*) and KIF3B (*H. sapience*). Klp67A was shown to be involved in spindle formation and chromosome segregation during meiosis and/or mitosis [Gandhi et al., 2004; Savoian et al., 2004]. KIF3B protein, homologous to MS-KIF18A, was demonstrated to take part in chromosomes tethering to the spindle pole and in chromosome movement. KIF3B, shown to mediate intraflagella transport in multiple ciliated cell types [Baker et al., 2003] and in epithelial cells, is involved in positioning of the tumor suppressor gene adenomatous polyposis coli (APC) [Jimbo et al., 2002]. Recent study has demonstrated that KIF18A is involved in chromosome segregation and spindle checkpoint in human cells [Zhu et al., 2005]. Kinesins are part of the cellular machinery and play a different role during the cell cycle; during mitosis they utilized the compression and movement of chromosomes while during interphase they may participate in trafficking of protein complexes/organelles.

In summary, our data presented the association between MS-KIF18A, ERα, and caveolin-1. These interactions are presumably involved in the estrogen non-genomic response and the activation of the ERK pathway in MBA-15 cells. Collectively, the observations indicate cooperation between ERα and MS-KIF18A. The study established the understanding of MS-KIF18A, a member of kinesin proteins, and its suggested role in signaling pathways by the trafficking of functionally related proteins.

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