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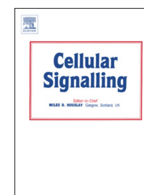

Methylosome protein 50 promotes androgen- and estrogen-independent tumorigenesis

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ABSTRACT

Methylosome protein 50 (MEP50) is a component of methylosome where MEP50 binds protein substrates and activates the oncogenic protein arginine methyl transferase 5 (PRMT5). MEP50 is also a coactivator for androgen receptor (AR) and estrogen receptor (ER), and transforms cells in the presence of androgen or estrogen. To extend the understanding of how MEP50 transforms cells, we investigated whether MEP50 could transform cells independent of AR and ER, and clarified whether PRMT5 could contribute to the MEP50-caused tumor formation. Microarray and Western blot analyses revealed the association of MEP50 with many human cancers including lung cancer. Knockdown of MEP50 retarded cell growth and migration in selected lung cancer cell lines, which expressed very low level of AR and ER and were insensitive to inhibitors of AR and ER. Moreover, overexpression of Myc-MEP50 enhanced cell transforming activities of 293T cells which are known lack of expression of AR and ER. Mechanistic analyses showed that MEP50 controlled G2 progression, upregulated cyclin-dependent kinase 1(CDK1)/cyclin B1, and activated the survival cascade Phosphoinositide 3-kinase (PI3K)/AKT. MEP50 promoted cell migration, and activated the cell migration pathways such as Ras-related C3 botulinum toxin substrate 1 (Rac1)/vasodilator-stimulated phosphoprotein (VASP), and forkhead box protein A2 (FOXA2)/slug/cadherin cascades. Further analyses revealed that MEP50 activated the survival factor PI3K through PRMT5-catalyzed dimethylation of PI3K. Collectively, it is concluded that MEP50 can transform cells independent of AR and ER, and PRMT5 has partial contribution to that process.

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1. Introduction

Methylosome protein 50 (MEP50), a WD-repeat containing protein with 342 amino acids, is identified during a search for the proteins interacting with the core component of methylosome, protein arginine methyltransferase 5 (PRMT5), in human cells [1]. The PRMT5/MEP50 complex is also reported in Xenopus [2] and Drosophila [3]. PRMT5 and MEP50 form a tetramer of heterodimers with four copies each of PRMT5 and MEP50, which acts as core components recruiting different binding partners such as a Polycomb group protein SUZ12 [4] or a methyl CpG binding domain protein methyl-CpG binding domain protein 2 (MBD2) [5] in a context dependent manner, and thereby enabling methylosome to specifically methylate a wide spectrum of substrates [6]. MEP50 is required for stimulating PRMT5 activity by binding and orienting the protein substrate to the PRMT5 catalytic site [7]. PRMT5/MEP50 methylates various histones and therefore suppresses expression of the genes involved in immune response [8], egg formation [2],

Abbreviations: MEP50, methylosome protein 50; PRMT5, protein arginine methyl transferase 5; AR, androgen receptor; ER, estrogen receptor; CDK1, cyclin-dependent kinase 1; PI3K, phosphoinositide 3-kinase; Rac1, ras-related C3 botulinum toxin substrate 1; VASP, vasodilator-stimulated phosphoprotein; FOXA2, forkhead box protein A2.

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differentiation [9] and tumor suppression [10]. Moreover, MEP50 is also engaged in numerous cellular reactions such as RNA processing,[1] oogenesis [11], DNA repair [12], and transcription regulation [13]. The interaction of MEP50 with 14-3-3, a scaffold protein that binds a multitude of functionally diverse signaling proteins [14], further suggests an involvement of MEP50 in more biological processes. For example, MEP50 is involved in the process of tumor formation [15–18]. Interestingly, PRMT5 is also known as an oncoprotein [19,20]. However, whether MEP50 and PRMT5 can work together to transform cells remains largely unknown.

Accumulated evidences reveal the involvement of MEP50 in cancer formation. MEP50 is reported as a colon cancer biomarker [21]. CDK4-induced phosphorylation of MEP50 increases the cell transforming activities of PRMT5 [22]. MEP50 is found as a cofactor of androgen receptor (AR) and estrogen receptor (ER) [16,18], and is demonstrated as an oncoprotein in AR- or ER-dependent and subcellular localization-dependent manners. MEP50 shows nuclear localization in breast cancer [18] and ovarian cancer [16], while cytoplasmic distribution of MEP50 is found in prostate cancer [17] and testicular tumor [15]. Overexpression of MEP50 mutant with forced localization to nucleus or cytosol can transform estrogen-dependent or androgen-dependent cells respectively. By contrast, introduction of ectopic MEP50 mutant forced to reside in cytosol or nucleus inhibits growth of estrogen-dependent or androgen-dependent cells respectively. Although documented reports point out the cell transforming activities of MEP50 in the cell context with functional AR or ER, yet it is unclear whether MEP50 is tumorigenic in AR- or ER-negative background. Moreover, it is also unknown whether absolute nuclear or cytosolic localization of MEP50 is always critical to its cell transforming activities.

To understand whether MEP50 can transform cells independent of AR or ER, and unravel the role of PRMT5 in MEP50-mediated tumor formation, we firstly show that MEP50 is associated with many human cancers including lung cancers where MEP50 is distributed evenly in whole cells. Knockdown of MEP50 reduces cell growth and cell migration of lung cancer cell lines A549, H1299 and H460 which express very low level of AR or ER and are insensitive to the ER or AR inhibitors. Furthermore, overexpression of Myc-MEP50 in an ER- and AR- negative cell line, 293T, stimulates cell proliferation in the conditions of normal serum, low serum, and poorly attaching environment, and enhances tumor formation in nude mice. Mechanistic studies reveal that MEP50 increases cell cycle progression by accelerating G2 phase and upregulating G2 phase cyclin/cyclin-dependent kinase (CDK), and encourages cell survival via activating Phosphoinositide 3-kinase (PI3K)/AKT. Moreover, MEP50 promotes cell migration through activating Ras-related C3 botulinum toxin substrate 1 (Rac1)/vasodilator-stimulated phosphoprotein (VASP) and (FOXA2)/slug/cadherins cascades. PRMT5 is selectively involved in the MEP50-stimulated activation of cell survival via methylating and activating the MEP50-bound PI3K. Taken together, we provide evidence showing that MEP50 can transform cells in an androgen- and estrogen-independent manner, and PRMT5 has partial contrition to the process.

2. Materials and methods

2.1. Chemicals, antibodies, RNA interference, and plasmids

Fetal bovine serum (FBS), Dulbecco's modified eagle medium (DMEM), RPMI1640, penicillin, streptomycin, and Lipofectamine™ were purchased from GIBCO-BRL (Bethesda, MD, USA). Nocodazole, polyhema, PF998425, and ICI182,780 were from Sigma Chemical Co. (St. Louis, MO). The antibodies against MEP50, anti-actin, -ER, and -AR were purchased from Santa Cruz Biotechnology (CA, USA). The antibodies against various CDKs, cyclins, and proteins involved in AKT or MAPK pathways were from Cell Signaling Technology Inc (MA, USA). The lentiviral shRNA production plasmids for MEP50, PRMT5, and Rac1 with the targeting sequence 5′GCAAAGTGAAGTCTTTGTCTT3′, 5′-GGCT CAAGCCACCAATCTATG-3′, and 5′-CCCTACTGTCTTTGACAATTA-3′ respectively, were obtained from the National RNAi core facility (Institute of Molecular Biology, Academia Sinica, Taiwan, R.O.C). The synthesized Rac1 siRNA and control siRNA were 5′-AAGGAGAUUGGUGCUGUA AAA-3′ [23] and 5′-UUCUCCGAACGUGUCACGUTT-3′ respectively. The Myc-tagged or HA-tagged MEP50 expression vectors were from Ken Yamamoto [4]. EGFP-MEP50 was obtained from ligation of the PCR-amplified MEP50 fragment to the pEGFP-C1 vector. EGFP-PRMT5 inactive mutant was a gift from Shilai Bao [24].

2.2. Tissue procurement and microarray analysis.

The study protocol in terms of the collection of patients' biopsies was approved by the Ethics Committee at Taichung or Taipei Veterans General Hospital. No patient had previously received any neoadjuvant treatment such as chemotherapy before surgery. All patients gave informed consent and signed the consent form individually. The study samples were obtained after surgery from a non-necrotic area of the tumor and from adjacent non-tumorous tissue from neighboring sites outside the tumor. Both tumor and adjacent non-tumor tissues were confirmed by pathologists (Supplemental Tables 1 and 2). The tissue samples were placed immediately in cryovials, frozen in liquid nitrogen, and stored at −80 °C until analysis by microarray and western blotting. Statistically significant differences in MEP50 between the pairwise tissue samples were analyzed by Wilcoxon signed-rank test. All analyses were carried out using the SPSS version 13.0 statistical software.

2.3. Cell cultures and transfection

All cell lines were purchased from the American Type Culture Collection, maintained in a humidified incubator at 37 °C in the presence of 5% CO_2 and were grown in DMEM medium (for 293T) or RPMI1640 (for A549, H460 and H1299) containing 5% (for 293T) or 10% (for A549, H460 and H1299) fetal bovine serum (FBS), 100 unit/ml penicillin and 100 μg/ml streptomycin. Sodium pyruvate and non-essential amino acids were added in RPMI 1640 medium. Transfection of cells was performed with Lipofectamine™ according to the manufacturer's instructions.

Table 1
Statistical analysis of differential expression of MEP50 in various primary cancers using NCBI GEO databases.

Disease	Probeset of MEP50	Variable	Sample number	P-value	GSE Acc#	References
Ovarian cancer	201420_s_at	Normal	12	0.05	GDS3592	36
		Cancer	12			
Lung cancer	201420_s_at	Normal	5	0.05[a]	GDS1312	37
		Cancer	5			
Astrocytoma	201420_s_at	Non-tumor	23	0.00[a]	GDS1962	38
		Astrocytoma	26			
Glioblastoma	201420_s_at	Non-tumor	23	0.00[a]	GDS1962	38
		Glioblastoma	81			

[a] Indicates the statistic significance of MEP50 expression between normal and cancerous tissues.

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