RESEARCH ARTICLE

MCPH1 Protein Expression in Normal and Neoplastic Lung Tissues

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Abstract

Lung cancer is the most common cause of cancer-related death in the world. The main types are small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC), the latter including squamous cell carcinoma (SCC), adenocarcinoma and large cell carcinoma. NSCLCs account for about 80% of all lung cancer cases. Microcephalin (MCPH1), also called BRIT1 (BRCT-repeat inhibitor of hTERT expression), plays an important role in the maintenance of genomic stability. Recently, several studies have provided evidence that the expression of MCPH1 gene is decreased in several different types of human cancers. We evaluated the expression of protein MCPH1 in 188 lung cancer and 20 normal lung tissues by immunohistochemistry. Positive MCPH1 staining was found in all normal lung samples and only some cancerous tissues. MCPH1-positive cells were significantly lower in lung carcinoma compared with normal tissues. Furthermore, we firstly found that MCPH1 expression in lung adenocarcinoma is higher than its expression in squamous cell carcinoma. Change in MCPH1 protein expression may be associated with lung tumorigenesis and may be a useful biomarker for identification of pathological types of lung cancer.

Keywords: Lung cancer - MCPH1 gene - protein expression - immunoistochemistry

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Introduction

Worldwide, lung cancer is the most common cause of cancer-related death in men and women, and is responsible for 1.38 million deaths annually (Ferlay et al., 2010). In China, lung cancer has the highest morbidity and mortality among malignant tumors around the country, which leads to 600 thousands deaths annually (He et al., 2013). lung cancer can be divided into two major types: non-small-cell lung cancer (NSCLC) and small cell lung cancer (SCLC) (Vinay et al., 2007). Non-small-cell lung carcinoma (NSCLC) is subdivided into three broad categories: pulmonary squamous cell carcinoma (SCC), pulmonary adenocarcinoma (AC) and large cell carcinoma (Dan et al., 2011). Prolonged cigarettesmoking is the most common cause of lung cancer (Biesalski et al., 1998), especially in China which has the largest number of smokers (300 million) in the world (Giovino et al., 2012). Non-small cell lung cancer accounts for about 80% of the total lung cancer cases in clinic (Fossella et al., 2003). Nearly 40% of lung cancers are adenocarcinoma, which usually originate in peripheral lung tissue. Squamous cell carcinoma accounts for about 30% of lung cancers. A hollow cavity and associated cell death are commonly found at the center of the tumor (Lu et al., 2010).

Primary microcephaly is an autosomal recessive genetic disorders disease, which often occurs in fetus during pregnancy, it is characterised by a severely diminished brain, MCPH1 is the first identified gene that associated with this disease (Hosseini et al., 2012; Shi et al., 2012). MCPH1 encodes microcephalin protein, which is also known as BRIT1 (BRCT-repeat inhibitor of hTERT expression), it was initially identified as a transcriptional receptor of human telomerase reverse transcriptase (Shi et al., 2012). MCPH1 gene mutation can cause premature chromosome condensation (PCC), then resulting in miscarriage or premature birth with cerebellar malformations fetus. Humans MCPH1 gene is located at 8p23.1, MCPH1 protein contains 835 amino acids with about 110 kDa of the molecular weight (Shi et al., 2013). There are three BRCT (Breast Cancer Carboxyl Terminal) domains in the MCPH1 protein, one of them is in N-terminus (N-BRCTs), the other two are in C-terminus (C-BRCTs) (Driscoll et al., 2006; Trimborn et al., 2006). Many important proteins that involved in DNA damage response and tumor suppression, such as BRCA1, BRCA2, 53BP1, XRCC1, Rad9, NBS1, and DNA polymeraseλ, are found that have BRCT domains (Aldecott, 2003; Goldberg et al., 2003; Lou et al., 2003; Stewart et al., 2003; Roy et al., 2011).

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Table 1. Clinicopathologic Characteristics of 188 Patients and Expression of MCPH1

Factors	N (%)
Gender	
Male	152 (80.9)
Female	36 (19.1)
Age (y)	
<50	41 (21.8)
≥50	147 (78.2)
Clinical stage	
I	106 (56.4)
II	46 (24.5)
III	34 (18.1)
IV	2 (1.0)
Histological type	
squamous cell carcinoma (SCC)	114 (60.6)
adenocarcinoma (AC)	64 (34.1)
adenosquamous carcinoma (ASC)	10 (5.3)
Pathological grading	
1	26 (13.8)
2	123 (65.4)
3	14 (7.5)
Ungraded	25 (13.3)
T classification	
T1	9 (4.8)
T2	149 (79.3)
T3	13 (6.9)
T4	17 (9.0)
N classification	
N0	128 (68.1)
N1	53 (28.2)
N2	6 (3.2)
N3	1 (0.5)
Expression of MCPH1	
Low	105 (55.9)
High	83 (44.1)

As far as we know, various of transcriptional regulatory proteins such as p53, RNApolII, RNA helicaseA, p300 and CtIP, are interacting directly or indirectly with BRCT domains of BRCA1. MCPH1 contains three BRCT domains, which suggests that BRCT-containing protein MCPH1 play a role in maintaining genome stability. The maintenance of genome stability needs perfect response to DNA damage in cells, which involves the activation of cell cycle checkpoint and the repair of damaged DNA, or, if the damage is unrepairable, the cell apoptosis or proliferates out of control (Mavrou et al., 2008; Branzei et al., 2011). There are two phosphatidylinositol 3-kinase-related kinases (PIKKs) play a crucial role in DDR: ATM (ataxia telangiectasia mutated) and ATR (ATM-Rad3-related), which activate a cascade of phosphorylation events to execute the DNA damage response (Bhattacharya et al., 2012). Recently, BRIT1/MCPH1 has been identified as an early ATM /ATR pathway mediator in the beginning of DNA damage, MCPH1 can co-localizes with numerous ATM/ATR pathway-associated proteins, including H2AX, MDC1, 53BP1, NBS1, p-ATM, ATR, p-RAD17, and p-RPA34, when cells were exposured to DNA damaging reagents (Bhattacharya et al., 2012). Now it has been confirmed that knockdown of MCPH1 gene could reduce the expression of BRCA1 and CHK1, and NBS1 can't be phosphorylated, then resulting in intra-S and G2/M checkpoint loss (Xu et al., 2004; Lin et al., 2005). Except to regulate the expression of BRCA1 and Chk1, MCPH1 also can prevent cells enter mitosis prematurely (Alderton et al., 2006). It had been reported that MCPH1 participated in modifying chromosome structure, MCPH1 can combine with Condensin II Complex, which involves in the process of chromatin condensation (Yamashita et al., 2011). MCPH1 had also been shown to interact with the chromatin remodeling complex SWI-SNF (Peng et al., 2009) and E2F1 (Yang et al., 2008) during DNA damage response.

From the above, we know that MCPH1 play a important role in maintaining genome stability. Indeedly, MCPH1 deficiency can lead to genomic instability in MCPH1-deficient cells (Gavvovidis et al., 2012) and MCPH1-/- mice (Liang et al., 2010; Zhou et al., 2013). We know that the genome in cancer cells is instable, in human, MCPH1 is located in 8p23.1, where loss of heterozigosity (LOH) of this site is common found in many types of human cancer. Recently, several studies had demonstrated that MCPH1 expression in several types of human cancer decreased, including breast cancer (Richardson et al., 2010), oral cancer (Venkatesh et al., 2013) and chronic myeloid leukemia (Giallongo et al., 2011). The abnormal expression of MCPH1 in human tumors supports the hypothesis that MCPH1 is a new tumor suppressor gene (Chaplet et al., 2006).

In this study, we investigated the expression of protein MCPH1 in normal lung tissues and lung carcinoma tissues by immunohistochemistry method. We investigated whether the reduction or alteration of MCPH1 protein expression associated with any clinicopathological characteristics in lung cancer.

Materials and Methods

Patients and control lung samples

The samples this study included non-small-cell lung carcinoma (NSCLC) diagnosed 188 patients and 20 normal lung tissues from healthy volunteers in the First Affiliated Hospital of Chongqing Medical University (Chongqing, China) between 2011 and 2013, all patients and volunteers had the same ethnic background. In the 188 patients diagnosed NSCLC, there were 152 males, 36 females, with a median age of 58.0 years-old (ranging from 30 to 77). The clinical characteristic of the NSCLC patients are described in detail in Table 1. All patients and healthy controls agreed to genetic testing, as approved by the hospital istitutional Review Board.

Immunohistochemical staining

Lung sections obtained from all healthy donors and patients were cut into 4 μm thick and 3 mm diameter sections to construct tissue microarrays. We used two selection criteria for tissue samples: 1) histologically proven diagnosis of lung cancer, 2) the proportion of tumor tissues exceeded 50% on micros copic slides. All sections were paraffin embedded following standard methods.

Rabbit polyclonal antibody to MCPH1 was used for immunohistochemistry (Abcam Ltd, Hong Kong: ab2612). According to the manufacturer instructions,

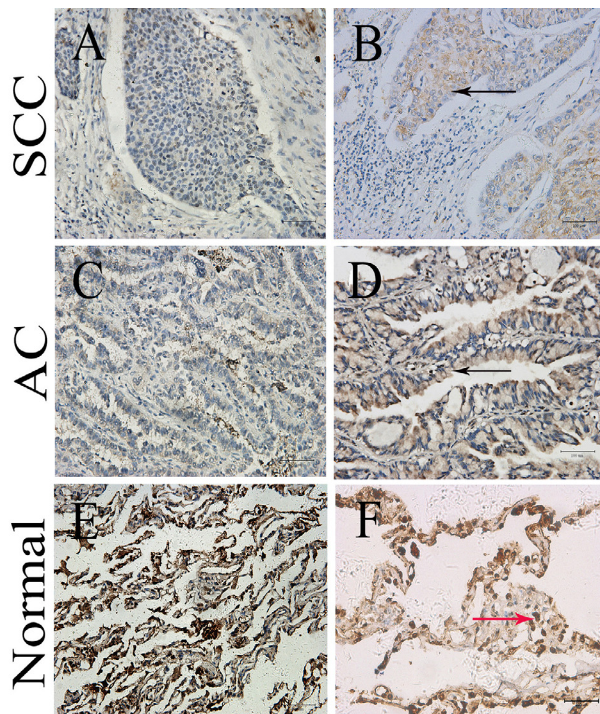

Figure 1. Expression Analysis of MCPH1 Protein in Lung Tissue Samples by Immunohistochemistry. A-F Immunohistochemistry analysis of MCPH1 expression in lung cancer tissue sections (n=188) including adenocarcinoma (AC), squamous cell carcinoma (SCC) and normal lung tissue sections (n=20) staining with anti-MCPH1 antibody. A, C MCPH1 is not expressed in AC and SCC . B, D MCPH1 showing low expression in NSCLC and mainly locating in the cytoplasm. E, F Normal lung tissue showing strong expression of MCPH1.E Normal lung tissue showing strong nuclear expression of MCPH1. MCPH1 was mainly expressed in the cytoplasm of SCC and AC cells (black arrows), alveolar cells showed strong nuclear expression of MCPH1 protein (red arrows). SCC: squamous cell carcinoma , AC: adenocarcinoma. B, E (×100), A, C, D, F (×200)

MCPH1 antibody, at the dilution of 1:300, has been shown to reliably recognize MCPH1 proteins in NSCLC and normal lung tissues by immunohistochemistry. Paraffin sections were processed as follow steps: 1) deparaffinized with Xylene, rehydrated with graded ethanol to distilled water, 2) subjected to 20 min in a microwave at 95°C in citrate buffer for antigen retrieval, 3) preincubated to 15 min in 3% H_2O_2 in citrate buffer to block the endogenous peroxidase, 4) thoroughly washed with washing buffer TBST (Phosphate Buffered Saline containing 0.05% Tween 20) in three 5 min cycles, 5) slides were then preincubated with 3% normal Goat serum albumin (GSA) for 30 min at 37°C, 6) incubated with 1:300 dilution of anti-MCPH1 antibody in PBS at 4°C for 16h, 7) rewarmed for 30 min at 37°C, 8) thoroughly washed with washing buffer TBST in three 5 min cycles, 9) incubated with biotinylated anti-rabbit secondary antibody for 30 min at 37°C, 10) thoroughly washed with washing buffer TBST in three 5 min cycles, 11) marked with streptavidin horseradish peroxidase (HRP) for 30 min at 37°C, 12) thoroughly washed with washing buffer TBST in three 5 min cycles, 13) for color reaction, stained with 3, 3'-diaminobenzidine (DAB) for 60 s. After detection,

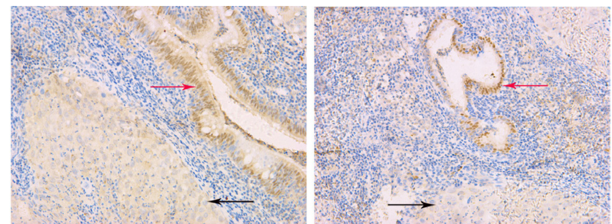

Figure 2. Expression of MCPH1 Protein in Cancerous Tissues and Normal Tissues Near the Cancer of the Lung (×100). MCPH1 was mainly expressed in the cytoplasm of SCC cells (black arrows), brochial epithelial cells showed strong nuclear expression of MCPH1 protein (red arrows)

all sections were counterstained with haematoxylin, dehydrated and then mounted in neutral balsam.

Evaluation of IHC staining

The quantification of degree of staining of MCPH1 protein expression was based on previous studies (Cao et al., 2010; Arthur et al., 2012), we used the intensity and extent of staining to evaluate MCPH1 expression. The entire tissue sections were observed under the optical microscope (100×) to assign scores. Each section was examined independently in a blinded fashion by two pathologists (Wei Cai, Dan Li). Extent of staining was scored as 0 (no staining), 1 (<25% of staining), 2 (26%-50% of staining), 3 (51%-75% staining), or 4 (75%-100% staining), according to the percentages of the positive staining areas in carcinomatous sections and entire section for the normal samples (Arthur et al., 2012). Meanwhile, intensity of staining was scored as 0 (no staining), 1 (light yellow staining), 2 (yellow staining), 3 (brown staining). The sum of the intensity and extent scores was used as the final staining score (0 to 7) of MCPH1. Section having a final staining score (<3) were grouped into low MCPH1 expression and those with scores (≥3) were grouped into high MCPH1 expression (Cao et al., 2010).

Statistical analysis

All statistical analyses were carried out by using the SPSS 16.0 statistical software package. Chi-square test of four-fold table were used to analyze the relationship between MCPH1 expression and clinicopathologic characteristics. $P<0.05$ was considered statistically significant.

Results

The decreased expression of MCPH1 in lung tissues

To determine the expression of MCPH1 in NSCLC tissues, we examined the expression MCPH1 protein in 188 paraffin-embedded NSCLC samples and 20 normal samples by immunohistochemical analysis. MCPH1 protein was detected in 128 of 188 (68.1%) NSCLC tissues and 19 of 20 (95%) normal samples. As shown in Figure 1, MCPH1 showed low expression in NSCLC samples (Figure1 A-D). MCPH1 protein was predominantly found to be strongly stained in normal sections (Figure 1 E-F). And MCPH1 was mainly expressed in the cytoplasm of

Table 2. Correlation Between the Clinicopathologic Features and Expression of MCPH1 Protein

Characteristics	MCPH1 (%)		p-Value
	Low expression	High expression	
Gender			0.125
Male	89(58.6%)	63(41.4%)	
Female	16(44.4%)	20(55.6%)	
Age (y)			0.166
<50	19(46.3%)	22(53.7%)	
≥50	86(58.5%)	61(41.5%)	
Clinical stage			0.142
I	59(55.7%)	47(44.3%)	
II	21(45.7%)	25(54.3%)	
III	23(67.6%)	11(32.4%)	
IV	2(100%)	0(0%)	
Clinical stage			0.068
I+II	80(52.6%)	72(47.4%)	
III+IV	25(69.4%)	11(30.6%)	
Histological type			0.002*
Malignant	105(55.9%)	83(44.1%)	
Normal	4(20%)	16(80%)	
Pathological grading			0.187
1	11(42.3%)	15(57.7%)	
2	76(61.8%)	47(38.2%)	
3	8(57.1%)	6(42.9%)	
Ungraded (25)			
Pathological grading			0.928
1+2	87(58.4%)	62(41.6%)	
3	8(57.1%)	6(42.9%)	
T classification			0.168
T1+T2	83(53.5%)	72(46.5%)	
T3+T4	22(66.7%)	11(33.3%)	
N classification			0.639
N0	68(57.1%)	51(42.9%)	
N1-3	37(53.6%)	32(46.4%)	
Cell type			0.002*
squamous cell carcinoma (SCC)	74(64.9%)	40(35.1%)	
adenocarcinoma (AC)	27(42.2%)	37(57.8%)	

cancer cells (Figure 1 B, D Figure 2 black arrows), normal lung tissue showed strong nuclear expression of MCPH1 protein (Figure 1 F, Figure 2 red arrows), which was consistent with recent reseach (Jo et al., 2013). These data suggest that MCPH1 show lower expression in NSCLC samples compared to normal samples, which indicates MCPH1 is related to the differentiation and metastasis of NSCLC.

The different expression in AC and SCC

Immunohistochemical determination of MCPH1 expression levels was statistically analyzed to identify an association with the clinicopathologic features of NSCLC (Table 2). As shown in Table 2, MCPH1 expression was significantly correlated with pathological type (P<0.05), and MCPH1 expression in non-small-cell lung cancer (NSCLC) was lower than in normal tissues (P<0.05). However, there was no significant correlation between MCPH1 expression and age, gender, clinical stage, pathological grading, T classifycation, or N classification. Our data firstly indicates that MCPH1 expression significantly correlated with local invasion and histological classification.

Discussion

The reports in recent years showed that MCPH1 expression was related to several female cancers, such as breast cancer (Richardson et al., 2011) and ovarian cancer (Brüning-Richardson et al., 2011). Compared with normal tissues, MCPH1 expression showed much lower in breast cancer and ovarian cancer, these findings indicated that MCPH1 plays a role in the progression of cancerization. Rai et al. found MCPH1 RNA and protein expression decreases significantly in the breast cancer cells (Rai et al., 2006). Moreover, Qin and van't Veer found a negative correlation between MCPH1 expression and breast cancer metastasis (Van't et al., 2002; Qin, 2002). Yu et al. showed that MCPH1 knockout mice were hypersensitive to γ-irradiation, although MCPH1−/− mice were able to survive to adulthood, they were growth retarded (Yu et al., 2010; Zhou et al., 2013). Zhang B et al. recently found that MCPH1 acted as a post- transcriptional regulator of p53 expression, and they demonstrated that knockdown of MCPH1 caused the oncogenic transformation of normal mammary epithelial cells (Zhang et al., 2013). In oral squamous cell carcinoma, MCPH1 was also downregulated at the transcript and protein levels, and it decreased cellular proliferation, cell invasion and tumor size in nude mice when MCPH1 was over expressed artificially (Richardson et al., 2010). Our previous studies found that over-expression of MCPH1 gene induced the apoptosis of HeLa cells in vitro (Hu et al., 2012). And MCPH1 was required for the expression of both BRCA1 and Chk1, MCPH1 knock-down leaded to reduced expression of BRCA1 and Chk1 in U2OS cells (Lin et al., 2005). In normal breast epithelial cells, MCPH1 was expressed as protein only in nucleus, but in breast cancer cells, It's expression was not only observed in nucleus but also in cytoplasm, The similar situation had been found in our study, the bronchial epithelial cells showed strong nuclear expression of MCPH1 protein, but MCPH1 was mainly expressed in the cytoplasm of lung cancer cells, the nuclear localization meaned that MCPH1 was consiten with the role in DNA repair and cell cycle regulation (Jo et al., 2013). The further research is needed to explain the cytoplasmic MCPH1 expression, preliminary researchs have shown that MCPH1 expression in cytoplasm is likely to be correlative with the progression and aggressiveness of breast cancer (Jo et al., 2013) and ovarian cancer (Brüning- Richardson et al., 2011). Those findings indicate that the MCPH1 played multiple roles in maintaining genomic instability, and cancer development, MCPH1 may function as a novel tumor suppressor gene.

In this study, we investigated expression of MCPH1 protein in normal lung tissues and lung carcinoma tissues by immunohistochemistry. The results firstly reveal that MCPH1 gene expression is downregulated in lung cancerous tissues compared with noncancerous tissues, indicating that MCPH1 gene participates in the development of lung cancer and could be a useful biomarker for identification of aggressive lung cancer, this conclusions are consistented with previous studies that MCPH1 expression was reduced in breast cancer, ovarian cancer and oral carcinoma. So the non-small-cell lung

carcinoma (NSCLC) is one of MCPH1-deficient cancers. We know the chromosome instability (CIN) is found commonly in most cancers, including lung cancer, and could be correlated with tumor grade and prognosis (Carter et al., 2006). CIN is thought to play a contributory role in tumor initiation and progression, the roles that MCPH1 platyed in the chromosome instability (CIN) can provide important mechanistic to understand the development and progression of lung cancer.

Interestingly, we also found MCPH1 expression in adenocarcinoma (AC) was higher than its expression in squamous cell carcinoma (SCC), the different expression in AC and SCC could make MCPH1 serve as a potential diagnostic biomarker in identifying histological type of lung cancer, and also provide an possibility in differentiation treatment of lung cancer. The traditional treatment of lung cancer is chemotherapy and radiotherapy, which cause strong side effects in the process of treatment, so the adjuvant therapy is goven more attention nowadays.

Recent research had demonstrated that poly (ADP-ribose) polymerase (PARP) inhibitors could kill BRCA1/2-deficient cells with high specificity, the mechanism for this specific cell-killing effect stems from a delicate synthetic lethal effect. PARP is an single-strand DNA breaks (SSBs) repair enzyme. In normal cells, the cells are well resistant against the DNA damage caused by PARP inhibitors, because there is a functional compensation effect from the HR-mediated DNA repair pathway, but the BRCA1/2-deficient cells, which are HR-repair-defective, can't cope with the increasing DNA damage, and then this cells show more hypersensitive to the DNA damage generated by PARP inhibitors (Farmer et al., 2005; McCabe et al., 2006; Balmaña et al., 2011; Basu et al., 2012). It had been proved that the knock down of MCPH1 can impair BRCA1-CHK1 DNA repair pathway and lead to defective HR repair (Lin et al., 2005). This findings provide a possibility that PARP inhibitors may be used as potential potent drugs to treat MCPH1-deficient cancers specifically. This adjuvant therapy strategy bringing some good news for patients with MCPH1- deficient cancers, such breast cancer and lung cancer. Recently, we used the traditional chemotherapeutic agent Cisplatin combined with PARP inhibitor 5-AIQ on the lung cancer cell lines A549, and found that 5-AIQ increased the sensitivity of A549 cells to cisplatin, suggesting that PARP inhibitors may be useful in the treatment of MCPH1-deficient cancers. In the future, we will test this idea in mammals and if possible, test it in clinic. It will also be worthwhile to detect the effect of PARP inhibitors on squamous cell carcinoma (SCC) and adenocarcinoma (AC) to find a potential way to treat the cancers with different expression in MCPH1.

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