

# hMOF reduction enhances radiosensitivity through the homologous recombination pathway in non-small-cell lung cancer

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Nan Li  
Guang-Wei Tian  
Ling-Rong Tang  
Guang Li

Department of Radiation Oncology,  
The First Affiliated Hospital of China  
Medical University, Shenyang, China

**Purpose:** Human males absent on the first (hMOF) is a histone acetyltransferase (HAT) and is responsible for acetylating histone H4 at lysine 16 (H4K16). Recent studies have indicated that hMOF is overexpressed in non-small-cell lung cancer (NSCLC) as an oncogene. The aim of this study is to profile the prognostic roles of hMOF in patients with unresectable stage III NSCLC undergoing definitive radiotherapy (RT) and in the radiosensitivity of human NSCLC cells.

**Materials and methods:** The expression of hMOF was detected in 24 normal and tumor-paired fresh-frozen NSCLC tissue samples. The immunohistochemistry was conducted, and the correlation of hMOF with clinicopathological parameters was studied in tissues from 90 patients with unresectable stage III NSCLC who underwent definitive RT. Radiation sensitivity was monitored using clonogenic assays in NCI-H1299 and A549 NSCLC cell lines with hMOF knockdown.

**Results:** hMOF was overexpressed in NSCLC tissues compared with non-cancerous tissues. Compared to patients with downregulated hMOF, upregulated hMOF was observed in 51.1% (46/90) of the patients, who showed a significantly worse 5-year survival rate (5.4% vs 22.9%,  $P=0.025$ ). hMOF expression was an independent prognostic factor of unresectable stage III NSCLC patients who underwent definitive RT. Silencing hMOF increased in vitro the sensitive enhancing ratio (SER) of NSCLC cell lines and downregulated the expression of phospho-ataxia telangiectasia mutated (p-ATM) and RAD51 after irradiation (IR).

**Conclusion:** Overexpression of hMOF predicts poor prognosis in patients with unresectable stage III NSCLC undergoing definitive RT. Downregulating hMOF might be a promising intervention to improve the outcome after RT.

**Keywords:** prognosis, NSCLC, hMOF, radiosensitivity, HATs

## Introduction

Non-small-cell lung cancer (NSCLC) is one of the most common causes of cancer-related death worldwide. Unresectable stage III NSCLC is the most common presentation among non-metastatic cases.<sup>1</sup> Radiotherapy (RT) is the definitive treatment modality of patients with unresectable stage III NSCLC. Despite concurrent chemoradiotherapy (cCRT) and some progress with the use of three-dimensional conformal radiation therapy (3D-CRT) and intensity-modulated radiation therapy (IMRT) in improving the clinical outcome, the 5-year survival rate is 16%.<sup>2-4</sup> Acquired radioresistance contributes to tumor recurrence and a poor prognosis; thus, identifying potential prognostic biomarkers and effective radiosensitizers has important clinical significance for improving the therapeutic efficacy of NSCLC patients.

Correspondence: Guang Li  
Department of Radiation Oncology,  
The First Affiliated Hospital of China  
Medical University, 155 Nanjing North  
Street, Heping District, Shenyang 110001,  
Liaoning Province, China  
Tel +86 24 8328 2004  
Email 13804058616@163.com

The histone modification status is dynamically regulated by chromatin-modifying enzymes in cells. Histone acetylation is a well-characterized epigenetic modification that is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs).<sup>5</sup> MOF is a member of the MYST family of HATs, which was first described in *Drosophila melanogaster* as an essential component of the X-chromosome dosage compensation male-specific lethal (MSL) complex and is responsible for the acetylation of H4K16 in the cell.<sup>6–11</sup> In human cells, MOF can form at least two distinct multiprotein complexes, MSL and non-specific lethal (NSL), and both complexes can acetylate H4K16 (acetylated H4K16 [H4K16ac]). In addition, MOF plays critical roles by acetylating non-histone substrates, such as p53, TIP5, and Nrf2.<sup>12–15</sup> MOF plays critical roles in chromatin stability, cell cycle, gene transcription, DNA damage repair, and early embryonic development.<sup>5,7,16,17</sup> Inconsistent hMOF expression and its corresponding acetylation of H4K16 have been found in various primary cancer tissues. Recent studies have shown that hMOF is frequently downregulated in breast cancer, medulloblastoma, ovarian cancer, renal cell carcinoma, colorectal carcinoma, and gastric cancer, whereas hMOF is upregulated in oral tongue squamous cell carcinoma and NSCLC.<sup>15,18,19</sup>

Several studies have reported that hMOF modulates proliferation and metastasis by regulating H4K16 acetylation in NSCLC cell lines and that hMOF predicts prognosis in patients with NSCLC.<sup>19,20</sup> hMOF depletion blocks the repair of DNA double-strand breaks (DSBs),<sup>5</sup> and the ability to repair DNA DSBs is related to cancer radiosensitivity.<sup>21</sup> Therefore, we hypothesized that hMOF expression levels and radiation resistance are related.

In this study, we aimed to investigate the role of hMOF in predicting the prognosis of patients with unresectable stage III NSCLC undergoing definitive RT and the correlation between hMOF and radiosensitivity in NSCLC cells.

## Materials and methods

### Patients and specimens

The study protocol was approved by the institutional review board of China Medical University. A total of 24 paired normal and tumor fresh-frozen NSCLC tissue samples were collected from the First Affiliated Hospital of China Medical University. The corresponding hMOF RNA and proteins were extracted. Between March 2008 and December 2013, 90 patients with unresectable stage III NSCLC who underwent curative 3D-CRT, IMRT with concurrent chemotherapy, sequential chemotherapy, or no chemotherapy at our

institution were recruited in our study. The chemotherapy regimens were cisplatin plus pemetrexed for non-squamous disease and carboplatin plus paclitaxel for all others. Fresh frozen lung resection biopsies were available. The prescribed dose was 60–66 Gy in 2.0 Gy daily fractions. The diagnosis was established using WHO morphological criteria. Tumor staging was performed according to the TNM classification of the seventh edition of the American Joint Committee on Cancer staging system.

### Ethics approval and consent to participate

This study was approved by the ethics committee of China Medical University. Written informed consent was obtained from all participants in the study. All experiments involving clinical samples were conducted in accordance with the Declaration of Helsinki.

### Real-time quantitative PCR and Western blot analysis

RNA was isolated with TRIZOL reagent (Thermo Fisher Scientific, Waltham, MA, USA). Real-time PCR (RT-PCR) was performed with a 7500 Real-Time PCR System (Thermo Fisher Scientific) using SYBR Green master mix (Takara, Dalian, China). GAPDH was used to standardize the quantity of hMOF transcripts. The relative expression of target genes was calculated using the  $2^{-\Delta\Delta Ct}$  method. The primer sequences are as follows: hMOF forward, 5'-TCTCACCATTCCCCGAAGA-3', hMOF reverse, 5'-TCCTTGGAGAAGTAGCCAACA-3'; RAD51 forward, 5'-CAGTGATGTCCTGGATAATGTAGC-3', RAD51 reverse, 5'-TTACCACTGCTACACCAAACATCAT-3'; and GAPDH forward, 5'-GAAGGTGAAGGTCGGAGTC-3', GAPDH reverse, 5'-GAAGATGGTGATGGGATTTTC-3'.

Proteins were extracted from cells and tissues lysed by RIPA lysis buffer and quantified using the Bradford method. Equal amounts of protein samples were transferred to polyvinylidene fluoride (PVDF) membranes (EMD Millipore, Billerica, MA, USA) and incubated overnight at 4 C with primary antibodies (anti-hMOF [Abcam, Cambridge, MA, USA; 1:500], anti- $\gamma$ -H2AX [Abcam; 1:1,000], anti-ataxia telangiectasia mutated [anti-ATM; Abcam; 1:1,500], anti-phospho-ATM [anti-p-ATM; Abcam; 1:2,000], anti-RAD51 [Abcam; 1:1,500], anti-H3 [Abcam; 1:3,000], anti-H4K16ac [Abcam; 1:1,000], anti-GAPDH [Abcam; 1:3,000], and anti- $\beta$ -actin [Boster, Wuhan, China; 1:3,000]).

The membranes were incubated with the corresponding secondary antibody for 2 h at room temperature. The target proteins on PVDF membranes were visualized

by ECL chemiluminescent substrate detection reagent (Beyotime, Shanghai, China) and measured using a DNR Imaging System (DNR, Jerusalem, Israel).

## Immunohistochemistry

Sections were deparaffinized using a series of xylene and rehydrated through graded alcohol to distilled water; then, antigen retrieval was performed in citrate buffer. Each section was incubated with a 1:80 dilution of anti-hMOF antibody and then with a biotinylated secondary antibody followed by streptomycin biotin peroxidase (SP) solution. Cells with brownish yellow particles in the cytoplasm were considered positive. Staining intensity was classified as follows: 0, no staining; 1, moderate staining; and 2, strong staining. The staining percentage was scored as follows: 1, 1%–24%; 2, 25%–49%; 3, 50%–74%; and 4, 75%–100%. The percentage and intensity scores were multiplied to result in a final hMOF score. hMOF overexpression was considered as a score of  $\geq 4$ .

## Cell culture and transfection

Human lung cancer cells NCI-H1299 (CCL-5803; American Type Culture Collection [ATCC], Manassas, VA, USA) and A549 (CCL-185; ATCC) cell lines obtained from Cell Bank of Type Culture Collection of Chinese Academy of Sciences were cultured in DMEM (Hyclone, Logan, UT, USA) containing 10% FBS (Clark, Richmond, VA, USA). Lentiviral hMOF-GV112-puromycin-shRNA (Genechem, Shanghai, China) and control lentiviral vector-GV112-puromycin shRNA were used for transfection according to the manufacturer's instructions and selected using puromycin. The transfected cells were labeled as hMOF-shRNA-H1299, Vector-H1299, hMOF-shRNA-A549, or Vector-A549.

## Irradiation (IR) conditions and clonogenic survival analysis

Culture flasks with H1299 and A549 cells were treated with X-ray IR using a linear accelerator (model: Varian Medical System) at a dose of 4 Gy when the cells reached 80% confluence. After IR, the cells were collected at the indicated time points.

Cells were passaged into single-cell suspensions, and a specific number of cells of each group were seeded into six-well plates. A single IR dose of 0, 2, 4, and 6 Gy was delivered to cells ( $n=200$  cells/each well for 0 and 2 Gy, 400 cells for 4 Gy and 600 cells for 6 Gy). After incubation for 14 days for colony formation, the cells were fixed in anhydrous ethanol and stained with crystal violet. The number of colonies

containing at least 50 cells was determined to calculate the plating efficiency (PE) and surviving fractions (SFs) by the formulae:  $PE = \text{colonies observed}/\text{number of cells plated}$  and  $SF = \text{colonies counted}/\text{cells seeded} \times (PE/100)$ . A multitarget single-hitting model was used to fit the dose survival curves. In the formula  $Y=1-[1-EXP(-k \times X)]^N$ , the parameters  $k$ ,  $N$ ,  $D_0$ ,  $D_q$  value were calculated ( $D_0=1/k$ ,  $D_q=\ln N \times D_0$ ), and the sensitive enhancing ratio (SER) was calculated by the formula,  $SER = (D_0 \text{ in the vector group}) / (D_0 \text{ in the hMOF-shRNA group})$ .

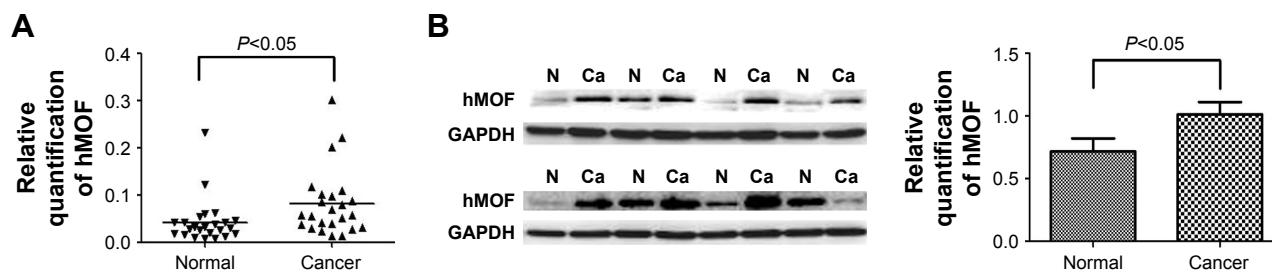
## Statistical analyses

SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. A  $\chi^2$  test was used to analysis correlations between hMOF expression and clinicopathological factors. Student's *t*-test was used to compare other data. A multitarget single hitting model was fit to the dose survival curve with GraphPad Prism 5.01 version software (GraphPad Software, Inc., La Jolla, CA, USA).  $P < 0.05$  was considered statistically significant.

## Results

### hMOF is overexpressed in NSCLC tissues and correlates with a poorer prognosis in NSCLC patients who underwent RT

RT-PCR and Western blot techniques were used to detect the mRNA and protein levels, respectively, of hMOF expression in 24 pairs of NSCLC tissues. Compared with non-cancerous tissues, the mRNA levels of hMOF were significantly increased (0.0812 vs 0.0419) ( $P < 0.05$ ) (Figure 1A). The overexpression of hMOF was also confirmed by Western blot (Figure 1B). Immunohistochemistry was performed in 90 cases of NSCLC tissues, and positive NSCLC cells displayed yellowish to brownish yellow granules in the nucleus (Figure 2A–D). hMOF expression was weak/negative in normal tissues, and hMOF overexpression was observed in 46 of 90 NSCLC tissues. We analyzed the correlation of hMOF expression with clinical factors of NSCLC patients and the prognosis of NSCLC patients. No significant association was found between hMOF and age, gender, smoking history, primary tumor (T) stage, or clinical stage, but hMOF was associated with lymph node (N) stage (Table 1). A Kaplan–Meier survival analysis revealed a significantly lower overall survival in patients with positive hMOF compared with those with negative expression (mean survival: 20 vs 28 months) ( $P=0.025$ ; Figure 2E). A multivariate Cox proportional hazards regression analysis



**Figure 1** hMOF is overexpressed in human lung cancer tissues.

**Notes:** Twenty-four pairs of NSCLC and corresponding non-cancerous tissues (>5 cm away from the cancer tissue) were studied to assess the role of hMOF. We used RT-PCR and Western blot techniques to detect the mRNA and protein levels, respectively, of hMOF in NSCLC tissues. **(A)** Compared with non-cancerous tissues, the mRNA levels of hMOF were significantly increased in NSCLC tissues. **(B)** Western blot analyses showed that hMOF protein expression in NSCLC tissues was higher than that in non-cancerous tissues.

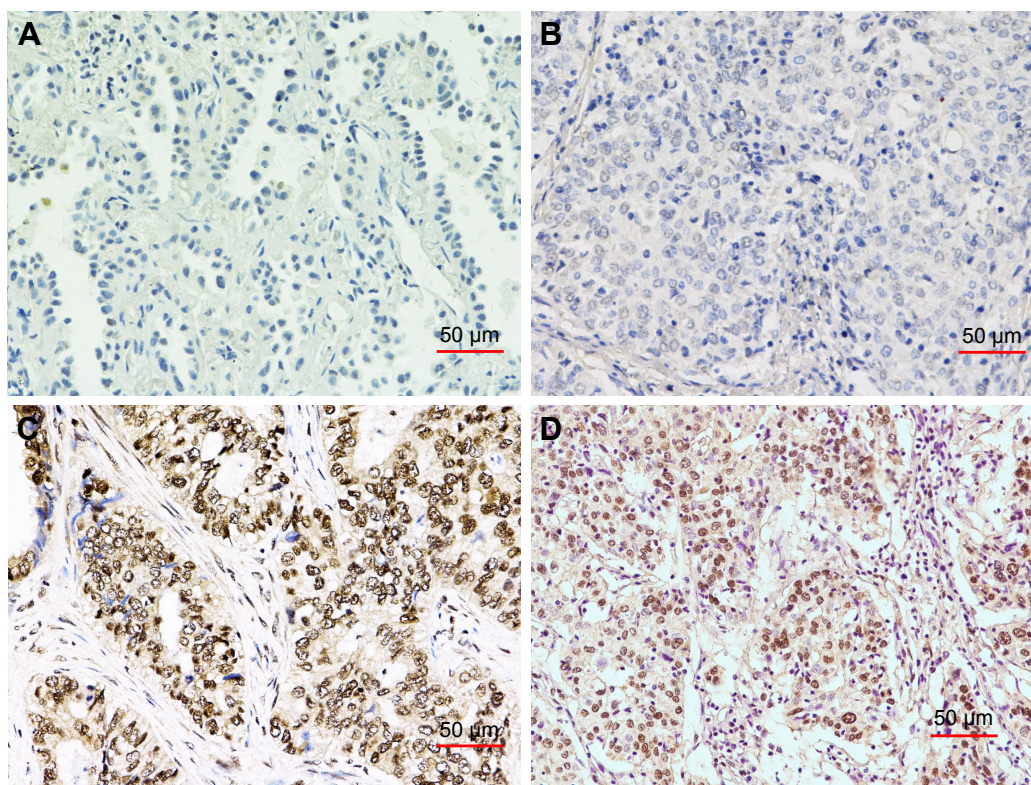
**Abbreviations:** hMOF, human males absent on the first; NSCLC, non-small-cell lung cancer; RT-PCR, real-time PCR.

indicated that hMOF expression, therapeutic regimen, and N stage were independent prognostic predictive factors for overall survival (Table 2).

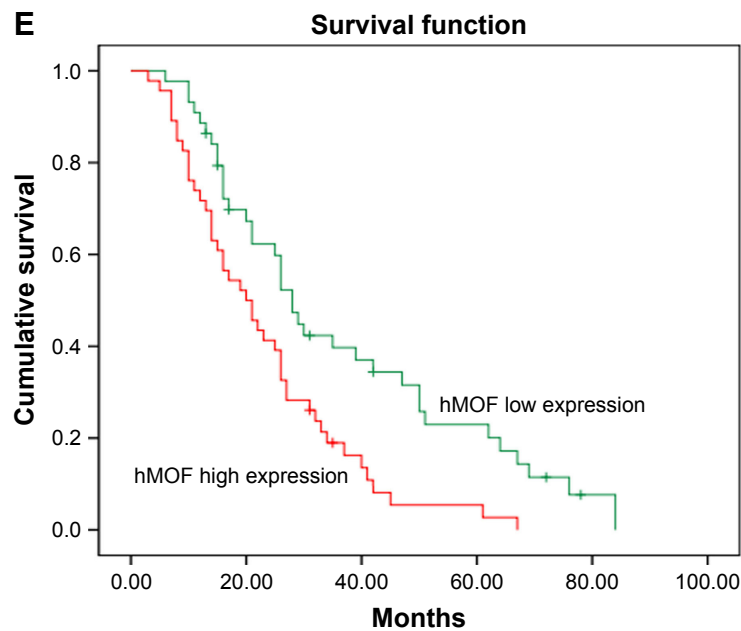
### Downregulation of hMOF sensitizes NSCLC cell lines to IR

As hMOF overexpression correlates with a poorer outcome in NSCLC patients who underwent RT, we investigated whether downregulated hMOF affected the response to RT. hMOF shRNA knockdown were performed in H1299 and

A549 cell lines, and the efficiency of shRNA knockdown was confirmed. The clonogenic assay is a gold standard for estimating radiosensitivity, and it was used to calculate survival curves. As shown in Figure 3, the number of formed cell clones decreased after exposure to graded doses of IR (0, 2, 4, and 6 Gy) compared with the Vector-H1299 group. Inhibition of colony-forming ability was more effective in the hMOF-shRNA-H1299 group under the same dose of IR, especially at the higher dose (6 Gy). Similar results were obtained for A549 cells. The dose survival curves were



**Figure 2** (Continued)



**Figure 2** Expression of hMOF protein in NSCLC tissues.

**Notes:** (A) Low expression of hMOF in adenocarcinoma of NSCLC tissues. (B) Low expression of hMOF in SCC of NSCLC tissues. (C) High expression of hMOF in adenocarcinoma of NSCLC tissues. (D) High expression of hMOF in SCC of NSCLC tissues. (E) Survival analysis of hMOF in NSCLC tissues. Magnification, 200 $\times$ .

**Abbreviations:** hMOF, human males absent on the first; NSCLC, non-small-cell lung cancer; SCC, squamous cell carcinoma.

**Table I** Distribution of hMOF status in NSCLC according to clinicopathological characteristics

Characteristics	Number of patients	hMOF low expression	hMOF over expression	P-value
Age, years				0.524
$\leq 61$	46	24	22	
$> 61$	44	20	24	
Gender				0.910
Male	70	34	36	
Female	20	10	10	
Smoking history				0.701
Non-smoking	35	18	17	
Smoking	55	26	29	
Pathological type				0.202
SCC	47	26	21	
Adenocarcinoma	43	18	25	
T stage				0.881
$T_{2-3}$	60	29	31	
$T_4$	30	15	15	
N stage				0.004
$N_{1-2}$	56	34	22	
$N_3$	34	10	24	
Clinical stage				0.094
III <sub>A</sub>	41	24	17	
III <sub>B</sub>	49	20	29	

**Abbreviations:** hMOF, human males absent on the first; NSCLC, non-small-cell lung cancer; SCC, squamous cell carcinoma.

**Table 2** Univariate and multivariate analyses of prognostic factors for overall survival using Cox proportional hazards regression model (N=90)

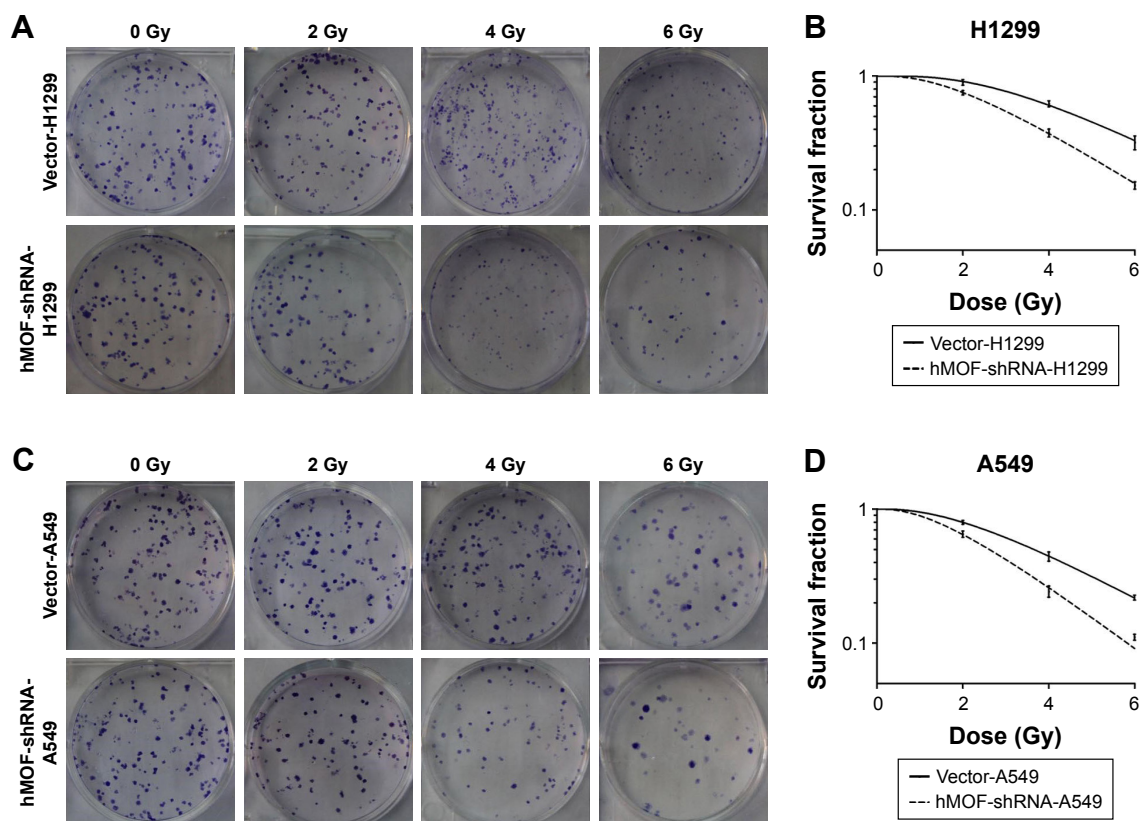
Characteristics	Univariate analysis		
	Hazard ratio	95% CI	P-value
Gender	1.005	0.594–1.701	0.985
Age	0.949	0.612–1.473	0.816
Dose	1.090	0.873–1.360	0.447
Smoking history	0.901	0.574–1.141	0.65
Pathological type	1.061	0.682–1.649	0.793
T stage	1.479	0.930–2.352	0.098
N stage	2.229	1.392–3.570	0.001
Clinical stage	1.446	0.920–2.273	0.110
Therapeutic regimen	1.809	1.330–2.459	<0.001
hMOF	2.038	1.282–3.239	0.003
Characteristics	Multivariate analysis		
	Hazard ratio	95% CI	P-value
hMOF	1.811	1.078–3.041	0.025
Therapeutic regimen	1.968	1.415–2.737	<0.001
N stage	1.799	1.073–3.015	0.026

**Abbreviation:** hMOF, human males absent on the first.

fit by using the multitarget single-hitting model, and the parameters  $k$ ,  $N$ ,  $D_0$ ,  $D_q$ ,  $SF$ , and  $SER$  are detailed in Table 3. After hMOF knockdown, we found that  $SER > 1$  in both H1299 and A549 cells at 1.22 and 1.34, respectively. These results indicated that downregulated hMOF could increase radiosensitivity in vitro.

## IR enhances hMOF-mediated acetylation of histone H4 at K16 and hMOF inactivation abrogates ATM and RAD51 activation

Previous reports indicated that hMOF and H4K16ac are critical for DDR and DSB repair<sup>5</sup> and that hMOF interacts with the ATM protein.<sup>8</sup> To explore the potential mechanism of enhanced radiosensitivity by hMOF reduction in NSCLC cells, we first detected the expression of hMOF and H4K16ac at 0, 1, 2, 4, and 8 h after 4 Gy X-ray treatment in NSCLC cell lines. As shown in Figure 4, the protein level of hMOF was consistent at different time points, whereas the expression of H4K16ac increased gradually and peaked at 4 h after



**Figure 3** hMOF reduction enhances the radiosensitivity of NSCLC cells in vitro.

**Notes:** (A and B) The number of formed cell clones decreased after exposure to graded doses of irradiation (0, 2, 4, and 6 Gy), compared with the Vector-H1299 group. The inhibition of colony-forming ability was more effective in the hMOF-shRNA-H1299 group under the same dose of irradiation, especially at the higher dose (6 Gy). (C and D) Similar results were obtained in A549 cells.

**Abbreviations:** hMOF, human males absent on the first; NSCLC, non-small-cell lung cancer.

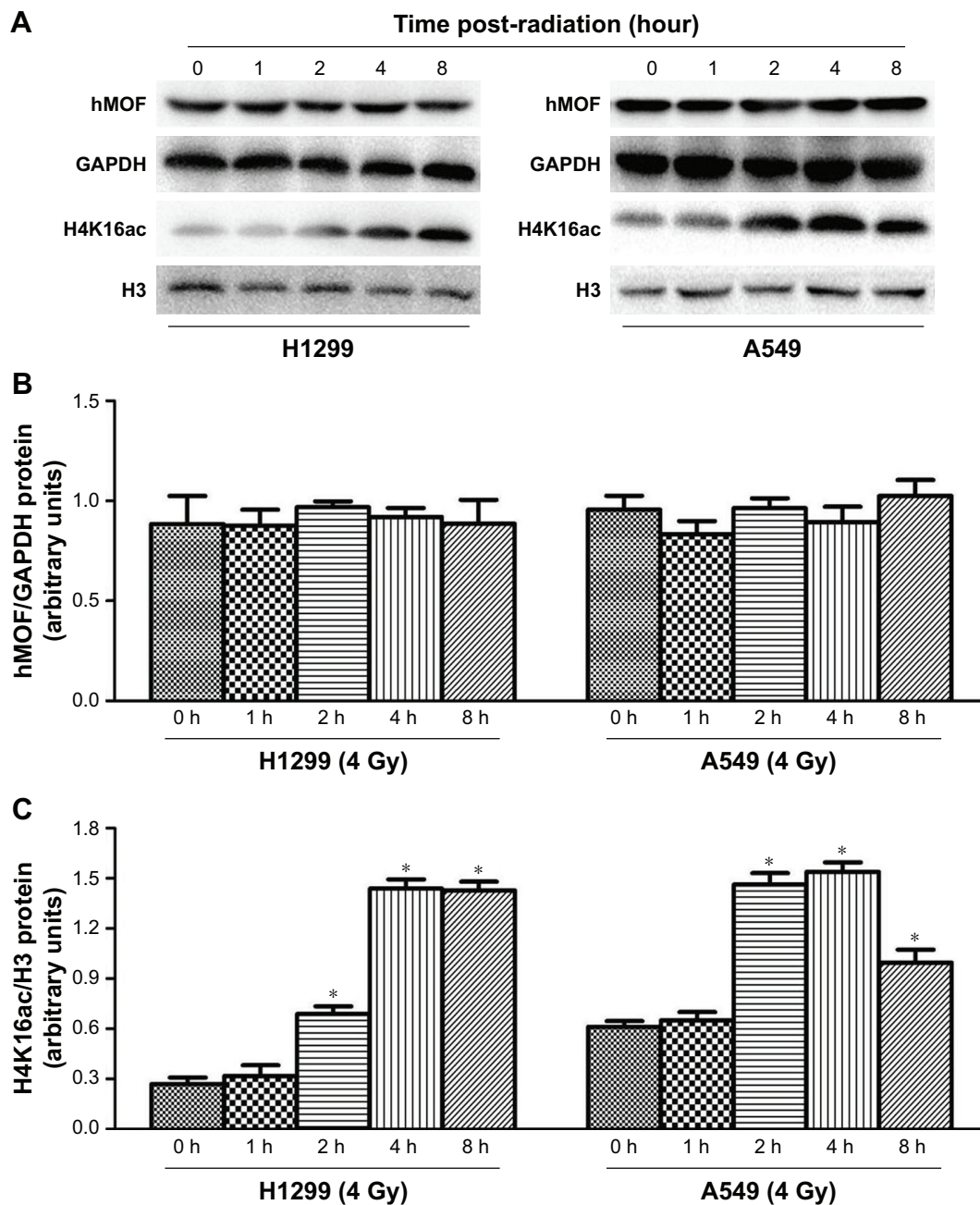
**Table 3** Relative biological parameters of the different cell groups after calculation using the multitarget single-hit model

	K	N	D <sub>0</sub>	D <sub>q</sub>	SER
Vector-H1299	0.3893	3.920	2.569	3.509	1.22
hMOF-shRNA-H1299	0.4734	2.841	2.112	2.205	
Vector-A549	0.4102	2.736	2.438	2.453	1.34
hMOF-shRNA-A549	0.5517	2.570	1.813	1.711	

**Abbreviations:** hMOF, human males absent on the first; SER, sensitive enhancing ratio.

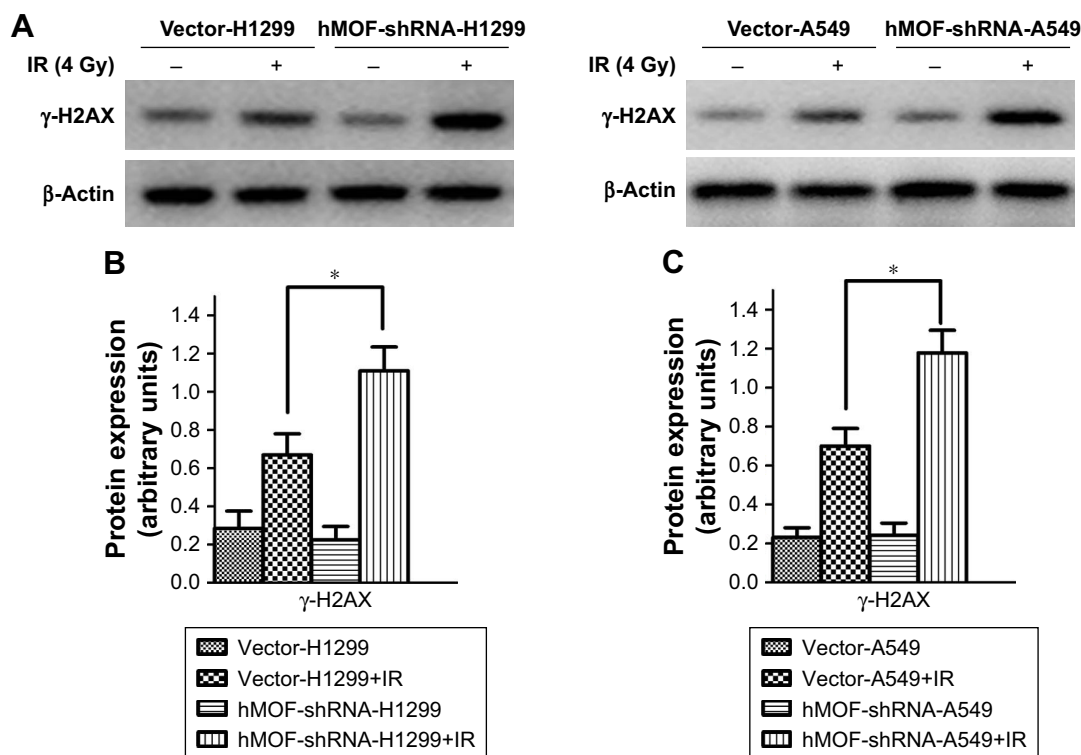
IR in both H1299 and A549 cell lines, indicating that IR enhanced hMOF-mediated H4K16ac and that 4 h after IR was the best reaction time.

To clarify whether the DSB level increased as a result of hMOF silencing, we performed a Western blot to test the expression of  $\gamma$ -H2AX, a sensitive and typical marker of DSB. The  $\gamma$ -H2AX protein was increased after IR, and a significant difference in  $\gamma$ -H2AX protein expression was found between

**Figure 4** Expression of hMOF and H4K16ac protein in NSCLC cells after IR.

**Notes:** The protein expression of hMOF and H4K16ac was detected at 0, 1, 2, 4, and 8 h after 4 Gy X-ray treatment in H1299 and A549 cell lines. At different time points after IR, the protein expression of hMOF was consistent (**A** and **B**), whereas H4K16ac protein expression increased gradually at 2 h and peaked at 4 h after IR in both cell lines (**A** and **C**). At 8 h, the H4K16ac level stayed consistent in the H1299 cell line but decreased in the A549 cell line, indicating that IR enhanced hMOF-mediated H4K16ac and that 4 h after IR was the best reaction time (\* $P < 0.05$ ).

**Abbreviations:** hMOF, human males absent on the first; IR, irradiation; NSCLC, non-small-cell lung cancer.



**Figure 5** Expression of  $\gamma$ -H2AX protein in NSCLC cells after IR.

**Notes:** Western blotting was performed to analyze  $\gamma$ -H2AX expression in H1299 and A549 cells transfected with an hMOF inhibitor at 4 h after 4 Gy X-ray treatment (**A**). Histograms show the relative expression levels of  $\gamma$ -H2AX in H1299 cells by grayscale analysis (**B**). Histograms show the relative expression levels of  $\gamma$ -H2AX in A549 cells by grayscale analysis (**C**). \* $p < 0.05$ .

**Abbreviations:** hMOF, human males absent on the first; IR, irradiation; NSCLC, non-small-cell lung cancer.

the vector-H1299+R and hMOF-shRNA-H1299+R cell groups (Figure 5). Similar results were obtained in A549 cells.

We then analyzed the protein levels of ATM, ATM phosphorylation (p-ATM), and RAD51, which are critical proteins of the recombination repair pathway, at 4 h after 4 Gy IR by Western blot. As shown in Figure 6A–C, the reduction of hMOF obviously decreased the acetylation of H4K16 and downregulated p-ATM and RAD51 expression compared with the control cells after IR in the H1299 and A549 cells ( $P < 0.05$ ). The results indicated that hMOF reduction enhanced radiosensitivity by affecting the homologous recombination (HR) repair pathway. RT-PCR was conducted to verify the mRNA change in RAD51 (Figure 6D) and showed that the mRNA expression was significantly decreased in the shRNA groups, indicating that hMOF may affect the protein expression of RAD51 at the level of transcription.

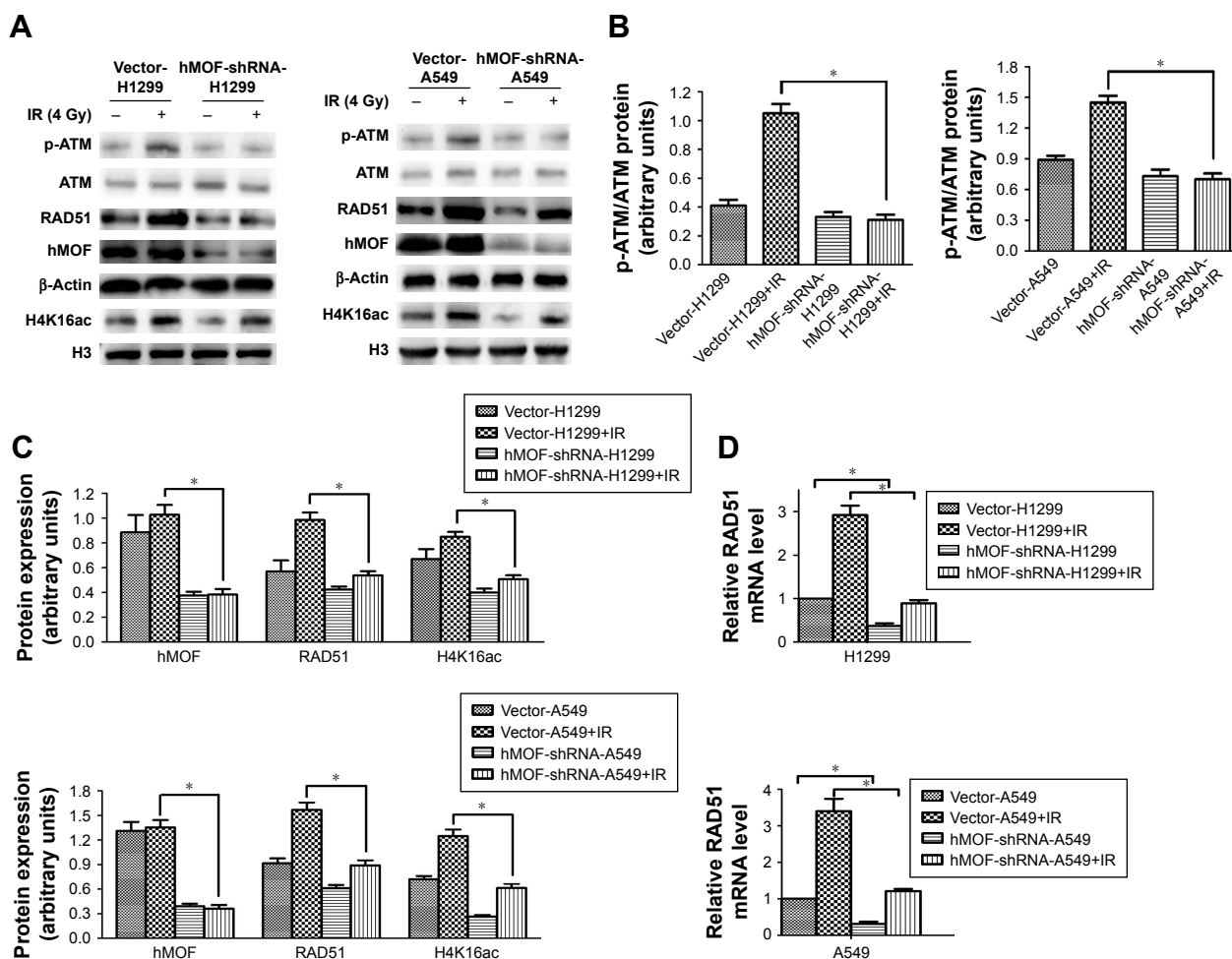
## Discussion

The loss of H4K16ac is a common hallmark of human cancer.<sup>22</sup> In recent years, hMOF was found to be overexpressed in NSCLC tissues and to have a potential oncogenic

role.<sup>15,18–20</sup> In this study, we demonstrated that the overexpression of hMOF predicted a poor prognosis and served as an independent prognostic factor in unresectable stage III NSCLC patients undergoing definitive RT. Importantly, we found that downregulated hMOF could increase radiosensitivity in both H1299 cells and A549 cells in vitro, which has not been previously studied.

High levels of hMOF were confirmed to be associated with metastasis, recurrence, tumor size, and disease stage.<sup>15</sup> In unresectable stage III NSCLC, we found that the expression of hMOF is associated with N stage, which may be a consequence of hMOF promoting the proliferation, migration, and adhesion of NSCLC cells.<sup>19</sup> The prognosis role of hMOF is inconsistent with other studies of NSCLC. Song et al<sup>18</sup> reported that hMOF overexpression was associated with good survival in patients with stage III NSCLC as an independent prognostic factor; high hMOF expression was associated with longer disease-free survival and overall survival. We found an opposite result in which the overexpression of hMOF predicted poor prognosis, which is consistent with the finding of another study.<sup>19</sup> We compared the patients included in our study with those in Song et al's study.





**Figure 6** IR enhances hMOF-mediated acetylation of histone H4 at K16 and hMOF inactivation abrogates ATM and RAD51 activation.

**Notes:** hMOF-shRNA-H1299, Vector-H1299, hMOF-shRNA-A549, and Vector-A549 cells were irradiated at a dose of 4 Gy in the IR groups. The same four groups were set as negative control groups without any treatment. At 4 h after IR, cells of each group were harvested to detect the protein expression of hMOF, H4K16ac, p-ATM, and RAD51. Representative Western blot results show that the expression levels of hMOF and ATM were consistent in the IR and control groups, whereas H4K16ac and RAD51 were increased by IR in all the cells (A and C). p-ATM was increased by IR in the Vector-H1299 and Vector-A549 groups, but there was no difference between the IR groups and control groups in the shRNA cell groups (A and B). The expression level of H4K16ac, RAD51, and p-ATM in the shRNA cell lines hMOF-shRNA-H1299 and hMOF-shRNA-A549 was clearly higher than those in their corresponding cell lines (B and C). These results indicated that hMOF reduction enhanced radiosensitivity by affecting the homologous recombination repair pathway. (D) RT-PCR analysis of RAD51 in all cell groups. RAD51 mRNA expression was significantly decreased in the shRNA groups, which indicated that hMOF may affect the protein expression of RAD51 at the level of transcription (\* $P < 0.05$ ).

**Abbreviations:** ATM, ataxia telangiectasia mutated; hMOF, human males absent on the first; H4K16ac, acetylated H4K16; IR, irradiation; NSCLC, non-small-cell lung cancer; p-ATM, phospho-ataxia telangiectasia mutated; RT-PCR, real-time PCR.

All 90 cases included in our study were patients with stage III, unresectable NSCLC, and the therapeutic regimen was RT with concurrent chemotherapy or sequential chemotherapy or no chemotherapy. In Song et al's study, the researchers recruited 129 patients with stage III NSCLC, but the regimen was not described. Neither of the two studies had a large sample size or was a randomized controlled trial; thus, large sample size studies are needed to confirm the conclusion.

cCRT is the standard treatment for patients with local, advanced NSCLC in clinical practice, and this treatment improved the 5-year overall survival rate from 10% to 16%.<sup>23</sup> Nevertheless, the acute, non-hematologic toxic effects, particularly esophagitis, are statistically significantly worse

with cCRT,<sup>23</sup> which results in more treatment breaks or treatment delays and impacts the clinical outcomes.<sup>24</sup> Moreover, patients with old age, bad performance status, or pre-existing comorbidities may not be able to tolerate cCRT due to the acute toxicity. The overexpression of hMOF predicted poor prognosis in unresectable stage III NSCLC patients undergoing definitive RT, and we speculated that hMOF might be associated with the radioresistance of NSCLC. The role of hMOF in facilitating drug resistance has been reported previously in NSCLC cells.<sup>15</sup> However, its involvement in the radioresistance of NSCLC remains unexplored. In this report, hMOF transfection and shRNA knockdown were performed in H1299 and A549 cell lines, and a better response

to IR was found in these cells than that in control cells. The underlying mechanism was explored through more in-depth research, and the expression of p-ATM and RAD51 was downregulated in NSCLC cells with depleted hMOF when treated by IR.

$\gamma$ -H2AX, a marker for DSBs, was positively associated with tumor radiosensitivity. Our results suggested that hMOF silencing could increase radiosensitivity through the upregulation of  $\gamma$ -H2AX expression. Pathways involved in the signaling and repair of DSBs are critical for protecting genomic stability and the outcome of radiation therapy.<sup>25</sup> HR is one of the major DSB pathways and is tightly associated with resistance to DNA-damaging agents. Research has shown that ATM signaling contributes to DSB repair not only for the initiation but also for the completion of DSB repair by HR in mammalian cells following exposure to IR and other DNA-damaging agents.<sup>26–28</sup> RAD51 is a highly conserved protein and a central player in DNA repair via HR. RAD51 foci formation is one of the most commonly used assays to functionally approach ongoing HR. In this study, we found a significant downregulation of p-ATM and RAD51 in cells transfected with hMOF shRNA after radiation. The mRNA levels of RAD51 were also decreased after silencing hMOF, and previous studies have demonstrated that p-ATM and RAD51 were critically involved in HR. Our results suggested that hMOF depletion might increase radiosensitivity through the HR pathway. The depletion of MOF blocks DSB repair by both the HR and NHEJ pathways (however, in our study, we did not find a significant change in the NHEJ pathway; data not shown).<sup>5</sup> Blocking the IR-induced increase in H4K16ac resulted in increased cell killing by decreasing ATM autophosphorylation, ATM kinase activity, and the phosphorylation of downstream effectors of ATM and DNA repair.<sup>8</sup>

## Conclusion

We demonstrated an important role for hMOF in the prognosis of patients with unresectable stage III NSCLC undergoing definitive RT. Downregulated hMOF increases radiosensitivity in vitro, which might be exploited to overcome radiation resistance and improve the efficacy of NSCLC RT.

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## Author contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

## References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin.* 2016;66(1):7–30. doi:10.3322/caac.21332
2. Jegadeesh N, Liu Y, Gillespie T, et al. Evaluating intensity-modulated radiation therapy in locally advanced non-small-cell lung cancer: results from the national cancer data base. *Clin Lung Cancer.* 2016;17(5):398–405. doi:10.1016/j.clcc.2016.01.007
3. Sher DJ, Koshy M, Liptay MJ, Fidler MJ. Influence of conformal radiotherapy technique on survival after chemoradiotherapy for patients with stage III non-small cell lung cancer in the national cancer data base. *Cancer.* 2014;120(13):2060–2068. doi:10.1002/ncr.28677
4. Auperin A, Le Pechoux C, Rolland E, et al. Meta-analysis of concomitant versus sequential radiochemotherapy in locally advanced non-small-cell lung cancer. *J Clin Oncol.* 2010;28(13):2181–2190. doi:10.1200/JCO.2009.26.2543
5. Sharma GG, So S, Gupta A, et al. MOF and histone H4 acetylation at lysine 16 are critical for DNA damage response and double-strand break repair. *Mol Cell Biol.* 2010;30(14):3582–3595. doi:10.1128/MCB.01476-09
6. Akhtar A, Becker PB. Activation of transcription through histone H4 acetylation by MOF, an acetyltransferase essential for dosage compensation in *Drosophila*. *Mol Cell.* 2000;5(2):367–375.
7. Gupta A, Guerin-Peyrou TG, Sharma GG, et al. The mammalian ortholog of *Drosophila* MOF that acetylates histone H4 lysine 16 is essential for embryogenesis and oncogenesis. *Mol Cell Biol.* 2008;28(1):397–409.
8. Gupta A, Sharma GG, Young CS, et al. Involvement of human MOF in ATM function. *Mol Cell Biol.* 2005;25(12):5292–5305.
9. Neal KC, Pannuti A, Smith ER, Lucchesi JC. A new human member of the MYST family of histone acetyl transferases with high sequence similarity to *Drosophila* MOF. *Biochim Biophys Acta.* 2000;1490(1–2):170–174.
10. Smith ER, Cayrou C, Huang R, Lane WS, Cote J, Lucchesi JC. A human protein complex homologous to the *Drosophila* MSL complex is responsible for the majority of histone H4 acetylation at lysine 16. *Mol Cell Biol.* 2005;25(21):9175–9188. doi:10.1128/MCB.25.21.9175-9188.2005
11. Taipale M, Rea S, Richter K, et al. hMOF histone acetyltransferase is required for histone H4 lysine 16 acetylation in mammalian cells. *Mol Cell Biol.* 2005;25(15):6798–6810. doi:10.1128/MCB.25.15.6798-6810.2005
12. Sykes SM, Mellert HS, Holbert MA, et al. Acetylation of the p53 DNA-binding domain regulates apoptosis induction. *Mol Cell.* 2006;24(6):841–851. doi:10.1016/j.molcel.2006.11.026
13. Sykes SM, Stanek TJ, Frank A, Murphy ME, McMahon SB. Acetylation of the DNA binding domain regulates transcription-independent apoptosis by p53. *J Biol Chem.* 2009;284(30):20197–20205. doi:10.1074/jbc.M109.026096
14. Zhou Y, Schmitz KM, Mayer C, Yuan X, Akhtar A, Grummt I. Reversible acetylation of the chromatin remodelling complex NoRC is required for non-coding RNA-dependent silencing. *Nat Cell Biol.* 2009;11(8):1010–1016. doi:10.1038/ncb1914
15. Chen Z, Ye X, Tang N, et al. The histone acetyltransferase hMOF acetylates Nrf2 and regulates anti-drug responses in human non-small cell lung cancer. *Br J Pharmacol.* 2014;171(13):3196–3211. doi:10.1111/bph.12661

16. Kind J, Vaquerizas JM, Gebhardt P, et al. Genome-wide analysis reveals MOF as a key regulator of dosage compensation and gene expression in *Drosophila*. *Cell*. 2008;133(5):813–828. doi:10.1016/j.cell.2008.04.036
17. Rea S, Xouri G, Akhtar A. Males absent on the first (MOF): from flies to humans. *Oncogene*. 2007;26(37):5385–5394. doi:10.1038/sj.onc.1210607
18. Song JS, Chun SM, Lee JY, Kim DK, Kim YH, Jang SJ. The histone Acetyltransferase hMOF is overexpressed in non-small cell Lung Carcinoma. *Korean J Pathol*. 2011;45(4):386–396. doi:10.4132/KoreanJPathol.2011.45.4.386
19. Zhao L, Wang DL, Liu Y, Chen S, Sun FL. Histone acetyltransferase hMOF promotes S phase entry and tumorigenesis in lung cancer. *Cell Signal*. 2013;25(8):1689–1698. doi:10.1016/j.cellsig.2013.04.006
20. Zhang S, Liu X, Zhang Y, Cheng Y, Li Y. RNAi screening identifies KAT8 as a key molecule important for cancer cell survival. *Int J Clin Exp Pathol*. 2013;6(5):870–877.
21. Sakata K, Someya M, Matsumoto Y, Hareyama M. Ability to repair DNA double-strand breaks related to cancer susceptibility and radiosensitivity. *Radiat Med*. 2007;25(9):433–438. doi:10.1007/s11604-007-0161-3
22. Fraga MF, Ballestar E, Villar-Garea A, et al. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nat Genet*. 2005;37(4):391–400. doi:10.1038/ng1531
23. Curran WJ Jr, Paulus R, Langer CJ, et al. Sequential vs. concurrent chemoradiation for stage III non-small cell lung cancer: randomized phase III trial RTOG 9410. *J Natl Cancer Inst*. 2011;103(19):1452–1460. doi:10.1093/jnci/djr325
24. McMillan MT, Ojerholm E, Verma V, et al. Radiation treatment time and overall survival in locally advanced non-small cell lung cancer. *Int J Radiat Oncol Biol Phys*. 2017;98(5):1142–1152. doi:10.1016/j.ijrobp.2017.04.004
25. Gachechiladze M, Skarda J, Soltermann A, Joerger M. RAD51 as a potential surrogate marker for DNA repair capacity in solid malignancies. *Int J Cancer*. 2017;141(7):1286–1294. doi:10.1002/ijc.30764
26. Pandita TK. A multifaceted role for ATM in genome maintenance. *Expert Rev Mol Med*. 2003;5(16):1–21. doi:10.1017/S1462399403006318
27. Shiloh Y. ATM and related protein kinases: safeguarding genome integrity. *Nat Rev Cancer*. 2003;3(3):155–168. doi:10.1038/nrc1011
28. Bakr A, Oing C, Kocher S, et al. Involvement of ATM in homologous recombination after end resection and RAD51 nucleofilament formation. *Nucleic Acids Res*. 2015;43(6):3154–3166. doi:10.1093/nar/gkv160

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