HAb18G/CD147 Promotes Radioresistance in Hepatocellular Carcinoma Cells: A Potential Role for Integrin β1 Signaling

Jiao Wu1, Yong Li1,2, Ya-Zheng Dang2, Hong-Xiang Gao2, Jian-Li Jiang1, and Zhi-Nan Chen1

Abstract

Radiotherapy has played a limited role in the treatment of hepatocellular carcinoma (HCC) due to the risk of tumor radioresistance. A previous study in our laboratory confirmed that CD147 interacts with integrin β1 and plays an important role in modulating the malignant properties of HCC cells. In this study, we further evaluated the role of CD147 in the radioresistance of HCC and as a potential target for improving radiosensitivity. Upon irradiation, the colony formation, apoptosis, cell-cycle distribution, migration, and invasion of SMMC-7721, CD147-knockout SMMC-7721, HepG2, and CD147-knockdown HepG2 cells were determined. A nude mouse xenograft model and a metastatic model of HCC were used to detect the role of CD147 in radioresistance in vivo. Deletion of HAb18G/CD147 significantly enhanced the radiosensitivity of SMMC-7721 and HepG2 cells, and knocking out HAb18G/CD147 in SMMC-7721 cells attenuated irradiation-enhanced migration and invasion. The knockout and antibody blockade of CD147 decreased the tumor growth and metastatic potentials of HCC cells under irradiation. CD147-deleted SMMC-7721 cells showed diminished levels of calpain, cleaved talin, active integrin β1, and decreased p-FAK (Tyr397) and p-Akt (Ser473) levels. FAK and PI3K inhibitors, as well as integrin β1 antibodies, increased the radiation-induced apoptosis of SMMC-7721 cells. Our data provide evidence for CD147 as an important determinant of radioresistance via the regulation of integrin β1 signaling. Inhibition of the HAb18G/CD147 integrin interaction may improve the efficiency of radiosensitivity and provide a potential new approach for HCC therapy. Mol Cancer Ther; 14(2); 553–63. © 2014 AACR.

Introduction

As the fifth most common malignant tumor, hepatocellular carcinoma (HCC) carries a poor survival rate. Most patients are not surgical candidates at the time of diagnosis because of vascular invasion, multifocality, and large tumor size, and liver shortage has been the rate-limiting step in the expansion of hepatic transplantation for patients with HCC (1, 2). Therefore, non-surgical therapies, including radiotherapy combined with other locoregional treatments, have been introduced to improve survival in patients with advanced HCC (3).

Radiotherapy has played a limited role in the treatment of HCC due to the low tolerance of the liver and the risk of tumor radioresistance (4). Despite the response of HCC to stereotactic body radiation therapy (SBRT) has been described for the past few years, and the fact that radiation can be delivered to tumors while avoiding damage to normal sensitive organs (5), the existence of radioresistant cells in HCC is remains an important reason for the local failure of radiotherapy (6, 7). Thus, understanding the mechanisms underlying radioresistance may provide an opportunity to develop more effective therapies.

As critical cell adhesion molecules that mediate the crosstalk between tumor cells and the signaling from the extracellular space into the cell, integrins participate in cell survival, invasion, metastasis, and some other malignant properties (8–11). Activation of the integrin pathway contributes to tumor radioresistance, and downstream focal adhesion kinase (FAK) and phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling have been linked to decreased radiation responsiveness in various malignant tumors, including head and neck cancer, pancreatic cancer, breast cancer, nasopharyngeal carcinoma, and glioblastoma (12–17). In human lung cancer cells, the upregulation of functional integrin β1 induced by ionizing radiation represents a prerequisite for metastasis (18). FAK knockout enhanced the radiosensitivity of HNSCC cells, and the downstream molecules of FAK, including paxillin, Akt1, and ERK1/2, were substantially dephosphorylated under FAK depletion (19). Irradiation enhances the invasiveness of HCC cells, which correlated with elevated MMP-9 expression and activated PI3K/Akt/NFκB signaling (7). Although several lines of evidence have suggested the contribution of the integrin pathway to radioresistance, little is known about its role in the radioresistance of HCC.

As a member of the immunoglobulin superfamily, CD147 is overexpressed in a number of epithelial cell–derived carcinomas and is associated with tumor development (20–23). In previous studies, we demonstrated that the interaction of HAb18G/CD147 with integrin β1 activates the downstream FAK/PI3K-Akt signaling pathway, subsequently enhancing the malignant properties of HCC cells.
and inhibiting the chemosensitivity of HCC cells (24, 25). Knocking out HAb18G/CD147 in HCC cells has been proven to result in p53 upregulation (26), and the functional p53 pathway could induce G1–G0 arrest or apoptosis upon radiation treatment (27). Given the correlations between apoptosis and radiosensitivity, we hypothesized that HAb18G/CD147 may enhance the radioresistance of HCC cells by interacting with integrin β1.

In this study, we demonstrated that HAb18G/CD147 contributes to the radioresistance of HCC cells. HAb18G/CD147 knockout and antibody blockade significantly enhanced radiosensitivity of HCC cells, and markedly attenuated irradiation-induced migration and invasion, as well as the tumor growth potential and metastatic potential. HAb18G/CD147 contributes to the integral β1 inside-out pathway and activates integrin β1. Meanwhile, HAb18G/CD147 interacts with integrin β1 and activates the outside-in pathway via FAK/PI3K-Akt signaling, thereby promoting HCC cell survival after irradiation. Inhibition of the HAb18G/CD147 integrin interaction may be a potential approach to improve the efficiency of radiosensitivity in HCC therapy.

Materials and Methods

Cell culture

Zinc finger nuclease technology was used to knock out HAb18G/CD147 in HCC SMMC-7721 cells, and the engineered cell line was named as K7721 (26). CD147 was stably knocked down in SMMC-7721 and HepG2 cells by short hairpin RNA (shRNA), and the cell lines were named as sh7721 (24) and shHepG2, respectively. The HCC cell lines SMMC-7721, K7721, sh7721, HepG2, shHepG2, and Huh7 were cultured in DMEM supplemented with 10% FCS and 1% penicillin/streptomycin. SMMC-7721 and HepG2 cells were obtained from the Institute of Cell Biology, Academic Sinica (Shanghai, China), and Huh7 cells were obtained from the JCRB Cell Bank. All cells have been authenticated by short tandem repeat profiling.

RNA extraction, reverse transcription, and real-time quantitative reverse transcription PCR

RNA extraction, reverse transcription, and real-time quantitative reverse transcription PCR (qRT-PCR) were performed as described previously (24). The primers used are listed in Supplementary Materials and Methods.

Irradiation procedure for cultured cells

Cells were irradiated using an X-ray (Elekta Precise Linac, the PLA 323 Hospital, Xi’an, China) or 60Co γ-ray facility (Radiation Center of Fourth Military Medical University, Xi’an, China) at room temperature. The doses applied in the experiments varied from 0 to 8 Gy.

Flow cytometric analysis

Flow cytometry was used to identify cell-cycle distribution and cell apoptosis and to detect CD147 and active integrin β1 expression in hepatoma cells. Detailed information is described in the Supplementary Materials and Methods.

HCC xenograft model of HCC

SMMC-7721 cells and K7721 cells were transfected with the lentiviral vector p-WPT-GFP to establish two GFP stably expressed cell lines named as 7721-GFP and K7721-GFP, respectively. An identical number of 7721-GFP cells and K7721-GFP cells (2 × 10⁶) were subcutaneously injected into the right flank of every nude mouse. X-ray treatment (Elekta Precise Linac, the PLA 323 Hospital, Xi’an, China; 4 Gy) was initiated when the tumors in 7721-GFP group achieved an average volume of approximately 100 mm³ (14 days). Mice were maintained under standard conditions and treated according to the institutional guidelines for animal care. The in vivo GFP signal was monitored at the indicated time points (2, 3, 4 weeks) using a Carestream In Vivo MS FX PRO imaging system. GFP excitation was provided by a 470 nm laser. Four weeks after inoculation, the mice were sacrificed. The tumor volume was calculated by length × (width)²/2. All animal procedures were performed in accordance with the Laboratory Animal Ethics Committee of Fourth Military Medical University (XYYLL-2013578; Xi’an, China).

HCC xenograft in an orthotopic intrahepatic model

7721-GFP and K7721-GFP cells were irradiated with X-rays at 4 Gy. A total of 24 mice were randomized into four groups (7721-GFP group, 7721-GFP + X-ray group, K7721-GFP group, K7721-GFP + X-ray group). The methodology used to create the orthotopic HCC model was previously described (24). For γ-ray irradiation, 7721-GFP cells mixed with Matrigel were injected into the left lobe of the liver in nude mice. In situ tumors achieved an average volume of approximately 100 mm³ at day 10 in a preliminary experiment with three mice. A total of 64 mice were randomized into 8 groups for different treatments. A humanized modified chimeric anti-HAb18G/CD147 antibody, HcHAb18 (metuzumab) was used to determine the effects of blocking HAb18G/CD147 on HCC cells. HcHAb18 had high affinity for the extracelular domain of CD147-II and strong antibody-dependent cell cytotoxicity. In preclinical experiments, HcHAb18 has been proven to be effective in preventing non–small cell lung carcinoma (data not published). From day 10 to 14, tumor-bearing mice were injected with different doses of HcHAb18 every 2 days via the tail vein. The same volume of 0.9% NaCl solution was injected into the contralateral control groups.

Invasion assay, wound healing assay, and colony-forming assay

7721 cells were cultured for the indicated days before Western blotting analysis of VEGF, MMP-2, MMP-9, and HAb18G/CD147.

Figure 1. High HAb18G/CD147 expression is associated with an enhanced ability to tolerate ionizing radiation. A, the inhibitory effect of X-ray irradiation on cell survival was determined by clonogenic assay, and phase-contrast images were taken (× 200). B, plating efficacy and survival curves of cells exposed to X-ray irradiation. C, apoptosis of hepatoma cells as determined by the Annexin V–FITC/PI binding assay 48 hours after 4 Gy irradiation. D, HAb18G/CD147 expression in HCC cells determined by Western blot and flow cytometry analyses. E, expression levels of the mRNAs encoding CD147, EGF, and VEGF after irradiation were measured by real-time PCR. F, SMMC-7721 cells were cultured for the indicated days before Western blotting analysis of VEGF, MMP-2, MMP-9, and HAb18G/CD147. G, SMMC-7721 cells (named 7721) and HAb18G/CD147-deleted SMMC-7721 cells (named K7721) were irradiated with 4 Gy X-rays and analyzed for VEGF, MMP-2, MMP-9, and HAb18G/CD147 6 days after irradiation. H, 7721 cells or X-ray-treated 7721 cells were subjected to an invasion assay and wound healing assay in the presence of VEGF-165 or an inhibitor of MMPs (marimastat). *P < 0.05; **P < 0.01; ***P < 0.001.
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**A**

Comparison of cell apoptosis and cell cycle distribution between 7721 and K7721 cells treated with or without X-ray radiation.

**B**

Comparison of cell apoptosis and cell cycle distribution between HepG2 and shHepG2 cells treated with or without X-ray radiation.

**C**

Graph showing the effect of X-ray radiation on the survival fraction of 7721 and K7721 cells.

**D**

Graph showing the effect of X-ray radiation on the survival fraction of HepG2 and shHepG2 cells.

**E**

Images showing the morphological changes of 7721 and K7721 cells treated with X-ray radiation.

**F**

Images showing the morphological changes of HepG2 and shHepG2 cells treated with X-ray radiation.
solution was used as a negative control. At day 14 and day 15, half the mice were irradiated with γ rays with a MASEP-SRRP Gamma System with an 8 mm collimator (the PLA 323 Hospital, Xi’an, China; 4 Gy). Mice were sacrificed under anesthesia when moribund or on day 43 for analysis of the survival and growth of tumors. Tumor growth was determined by GFP imaging using a Kodak Image Station 4000 MM PRO Digital Imaging System. The volume of in situ tumors was measured and the number of macroscopic metastases was counted.

Statistical analysis
Unless otherwise stated, data were obtained from three independent experiments. The results were expressed as the mean ± SD. Student t-test and one-way ANOVA were used to compare the mean values. Survival rates were compared using Kaplan–Meier survival curves. Statistical significance was set at P < 0.05.

Results
Effects of X-ray irradiation on the survival and apoptosis of HCC cells with different HAB18G/CD147 levels
Three HCC cell lines, HepG2, Huh7, and SMMC-7721, were exposed to X-ray irradiation at doses varying from 0 to 8 Gy. Cell survival was determined by a standard clonogenic assay (Fig. 1A and B), and cell death was analyzed with Annexin V–FITC/PI staining (Fig. 1C). According to the single-hit multitarget model, D0 values were determined to be 2.38 Gy for Huh7 cells, 2.80 Gy for HepG2 cells and 2.92 Gy for SMMC-7721 cells (Fig. 1B). The relative apoptotic rates, shown in Fig. 1C, confirmed that apoptosis was significantly induced in Huh7 cells after exposure to 4 Gy X-ray radiation. These results indicated higher radioresistance in SMMC-7721 and HepG2 cells compared with Huh7 cells. Flow cytometry studies revealed that CD147+ cells accounted for 89.94% ± 0.20% of HepG2 cells, 21.81% ± 2.55% of Huh7 cells, and 85.07% ± 0.76% of SMMC-7721 cells (Fig. 1D), which is in accordance with their abilities to tolerate ionizing radiation, demonstrating that HCC cells with high levels of HAB18G/CD147 could tolerate cell death and had increased resistance to radiotherapy.

X-ray radiation induced the upregulation of HAB18G/CD147 and molecules related to angiogenesis and metastasis
As shown in Fig. 1E, the mRNA levels of BSG (encoding CD147), VEGF, and EGF were significantly increased in HepG2, Huh7, and SMMC-7721 cells after exposure to low-dose X-ray radiation. The dose-dependent increase in expression levels of these molecules was already apparent after 3 days and reached a maximum after 9 days. Western blot analyses of HAB18G/CD147, VEGF, MMP-2, and MMP-9 in SMMC-7721 cells after irradiation are shown in Fig. 1F. It is evident that in SMMC-7721 cells that are resistant to irradiation, low-dose X-ray radiation promoted the expression of HAB18G/CD147, as well as that of genes related to angiogenesis and metastasis. As shown in Fig. 1F, knocking out HAB18G/CD147 in SMMC-7721 cells inhibited the upregulation of VEGF, MMP-2, and MMP-9 under irradiation. We also showed that VEGF and MMPs increased the invasive and migratory potentials of SMMC-7721 cells (Fig. 1G and H), which may promote tumor progression under low-dose radiation. These results demonstrate a potential role of upregulated HAB18G/CD147 in processes contributing to HCC radioresistance.

CD147 deletion enhanced the radiosensitivity of HCC cells
To further investigate the role of HAB18G/CD147 in radioresistance, CD147 was stably knocked out or knocked down by the zinc finger nuclelease technology or shRNA in SMMC-7721 and HepG2 cells, respectively, and the cells were assessed for apoptosis and cell-cycle redistribution by flow cytometry. The exposure of K7721 and shHepG2 cells to 4 Gy radiation resulted in more Annexin V-positive cells (K7721: early apoptosis, 34.7%; shHepG2: early apoptosis, 27.1%) compared with SMMC-7721 cells (early apoptosis, 8.2%) and HepG2 cells (early apoptosis, 5.6%) (Fig. 2A and B). As shown in Fig. 2C, the percentage of SMMC-7721 cells arrested at G0–G1 phase significantly decreased after irradiation (P < 0.05), whereas the percentage of cells in S-phase increased (P < 0.01). Compared with SMMC-7721 cells, K7721 cells treated with the same dose of X-ray irradiation presented an increased percentage of cells in G0–G1 phase (Fig. 2C, from 50.18% ± 1.58% to 89.65% ± 3.47%, P < 0.001) and a reduced percentage in S-phase (Fig. 2C, from 41.82% ± 1.58% to 2.01% ± 2.23%, P < 0.001). This finding indicates that cell-cycle distribution was significantly blocked in G0–G1 phase when CD147-deleted SMMC-7721 cells were treated with X-ray irradiation. The percentage of cells in S-phase decreased and that in G0–G1 phase increased in shHepG2 compared with HepG2 cells. shHepG2 cells treated with X-ray irradiation presented a reduced percentage of cells in G2–M phase (from 56.09% ± 0.16% to 29.93% ± 3.61%, P < 0.001) and an increased percentage in G0/G1 phase (from 29.86% ± 1.29% to 49.20% ± 9.93%, P < 0.001) compared with HepG2 cells (Fig. 2D). After exposure to X-ray irradiation at doses varying from 0 to 8 Gy, the survival fractions of SMMC-7721 and K7721 cells were determined, as shown in Fig. 2E. D0 values were determined to be 2.97 Gy for SMMC-7721 cells and 1.50 Gy for K7721 cells. In addition, the clones formed by K7721 cells contained fewer cells than clones formed by SMMC-7721 cells, implying slower cell division. Similar results were observed in HepG2 and shHepG2 cells (Fig. 2F). D0 values were determined to be 2.77 Gy for HepG2 cells and 1.92 Gy for shHepG2 cells. These results imply that HAB18G/CD147 deletion enhances radiosensitivity in SMMC-7721 and HepG2 cells by inducing cell apoptosis and cell-cycle arrest in G0–G1 phase.

CD147 deletion attenuates the irradiation-enhanced invasion and migration of HCC cells
To determine the effects of irradiation on HCC cell motility and invasion, SMMC-7721 and K7721 cells were treated with or
show in Fig. 4. Irradiation exhibited a strong inhibitory effect on the intrahepatic metastasis of K7721-GFP cells \( (P < 0.01) \) but effectively enhanced the metastasis of 7721-GFP cells compared with that of the nonirradiated 7721-GFP group \( (P < 0.05) \). PET-CT scanning displayed an inhomogeneous uptake of \(^{18}\)F-FDG in the livers of mice inoculated with irradiated 7721-GFP cells. In contrast, in mice implanted with irradiated K7721-GFP cells, no liver lesions were observed (Supplementary Fig. S1). These results show that the deletion of HAb18G/CD147 attenuated the promotion of hepatic metastasis induced by irradiation.

**HcHAb18 increased the radiosensitivity of HCC cells in an orthotopic intrahepatic model**

To block CD147 in the membranes of HCC cells, we pretreated SMMC-7721 cells with a humanized modified chimeric anti-HAb18G/CD147 antibody, HcHAb18 (metuzumab). Because both X-knife and \( \gamma \)-knife therapies have been developed to treat patients with liver cancer in China, we combined \( \gamma \)-knife therapy with HcHAb18 for the treatment of HCC in the orthotopic intrahepatic mouse model. After exposure to \( \gamma \)-ray irradiation at doses varying from 0 to 8 Gy, the survival fractions of cells were determined \( (\text{Fig. 5A and B}) \). Our results showed that HcHAb18 treatment enhanced the radiosensitivity of HCC cells in a dose-dependent manner. Compared with treatment with \( \gamma \)-ray irradiation or HcHAb18 alone, the combination of \( \gamma \)-ray irradiation and HcHAb18 resulted in a significant decrease in the survival rate \( (\text{Fig. 5B}) \). We further constructed an orthotopic intrahepatic HCC model in nude mice to examine the effects of HcHAb18 in radiotherapy. As shown in Fig. 5C–E, although \( \gamma \)-ray radiation alone exhibited a strong inhibitory effect on the growth of \textit{in situ} HCC tumors \( (P < 0.01) \), the number of intrahepatic metastases was increased \( (P < 0.05) \). HcHAb18 suppressed tumor growth in a dose-dependent manner. In addition, there was a significant inhibition of \textit{in situ} tumor growth and intrahepatic metastasis in animals treated with the combination of high-dose HcHAb18 (50 mg/kg) and radiotherapy compared with those treated with \( \gamma \)-ray radiation or high-dose HcHAb18 alone. There were marked differences between groups in terms of tumor growth and metastasis.
variations in the survival of mice between all of the groups (Fig. 5F, \( P = 0.0027 \)). In comparison with both the saline-treated control group with \( \gamma \)-ray radiotherapy and the 50 mg/kg HcHAb18-treated group, mice treated with a combination of 50 mg/kg HcHAb18 and radiotherapy showed prolonged survival (Fig. 5F). These results suggest that the antibody blockade of CD147 markedly inhibited the tumor growth and intrahepatic metastasis of HCC cells induced by radiation and improved the therapeutic effect of radiotherapy.

Integrin signaling is involved in the radioresistance of HCC cells induced by HAb18G/CD147

To investigate the role of the HAb18G/CD147 integrin interaction in the radioresistance of HCC cells, we examined HAb18G/CD147 expression and integrin activation by flow cytometry. As shown in Fig. 6A, 90.4% of SMMC-7721 cells but only 18.3% of sh7721 cells were CD147 positive. Accordingly, active integrin \( \beta \)1 levels of sh7721 cells were much lower (8.2%) compared with those of SMMC-7721 cells (28.7%). Among the active integrin \( \beta \)1-positive subpopulation of SMMC-7721 cells, approximately 98.3% cells were positive for CD147 expression. These results indicated the association of CD147 expression and integrin \( \beta \)1 activation. Immunoblotting showed that CD147 knockdown resulted in decreased levels of calpain and cleaved talin (Fig. 6B). These results demonstrated a crucial role of CD147 in mediating integrin \( \beta \)1 activation via inside-out integrin signaling. A previous study found that talin cleavage by calpain promotes its binding to the integrin cytoplasmic domain, thereby enhancing integrin activation. As shown in Fig. 6C, suppressing calpain and talin expression in SMMC-7721 cells using transient siRNA silencing resulted in dramatically reduced FAK phosphorylation (Tyr397).

Figure 4.
Effects of HAb18G/CD147 expression and irradiation on the tumorigenesis and metastasis of HCC xenografts. A–C, 7721-GFP and K7721-GFP cells were subcutaneously injected into the right flanks of nude mice. The growing tumors received 4 Gy radiation or no treatment 14 days after inoculation. A, images of tumors were acquired by GFP imaging at the indicated time points (from 2 to 4 weeks). Signals were displayed as pseudocolor heatmap images overlaid on gray scale X-ray images. B, mice were sacrificed 4 weeks after inoculation. Volumes of the xenografts were evaluated by measuring in two directions with a caliper. C, the final tumor weight at sacrifice (\( n = 6 \)). D–F, GFP-expressing HCC cells were irradiated with 4 Gy X-rays and subjected to orthotopic implantation 48 hours after irradiation. D, representative examples of livers with \( \text{in situ} \) tumors and intrahepatic metastases are shown, as determined by the GFP signal. Signals were displayed as pseudocolor heatmap images (white, most intense). E, volume of \( \text{in situ} \) tumors (\( n = 6 \)). F, number of intrahepatic metastases (\( n = 6 \)). *, \( P < 0.05 \); **, \( P < 0.01 \); ***, \( P < 0.001 \).
demonstrating that calpain and talin facilitate integrin outside-in signaling.

The expression levels of both p-FAK (Tyr397) and p-Akt (Ser473) were significantly diminished in K7721 cells compared with those in SMMC-7721 cells (Fig. 6D). Moreover, we observed slightly increased FAK and Akt phosphorylation in SMMC-7721 cells irradiated with X-rays. Because the deletion of CD147 enhanced the radiosensitivity of HCC cells, these results imply the involvement of the FAK-PI3K/Akt pathway in the radioresistance of HCC cells mediated by CD147. We also found upregulated WT p53 protein expression in K7721 cells compared with SMMC-7721 cells (Fig. 6D).

To explore the role of the FAK-PI3K/Akt pathway in radioresistant HCC cells, we tested the abilities of FAK and PI3K inhibitors to increase the radiosensitivity of SMMC-7721 cells. Signaling inhibition by these inhibitors was confirmed, as shown by decreased FAK and Akt phosphorylation (Fig. 6D). Radiation caused early apoptosis at 8.85%, whereas combined treatment of the cells with a signaling inhibitor and irradiation significantly enhanced the induction of apoptosis by 4.37 times and 3.68 times.
for the FAK inhibitor and PI3K inhibitor, respectively (Fig. 6E). We also observed enhancing effects of the integrin blocking antibody on the levels of apoptosis, with or without irradiation (Fig. 6E). These results confirmed the involvement of the integrin–FAK–PI3K/Akt pathway in the radioresistance of HCC cells. Because deletion of HAb18G/CD147 significantly inhibited the activation of integrin–FAK–PI3K/Akt signaling and enhanced the radiosensitivity of HCC cells, we have reasons to believe that HAb18G/CD147 mediates the radioresistance of HCC cells via the integrin–FAK–PI3K/Akt signaling.

Discussion

In this study, we demonstrated the role of HAb18G/CD147 in mediating the radioresistance of HCC cells. Cells with higher HAb18G/CD147 levels showed stronger resistance to sublethal irradiation, providing a possibility for exploring the function of HAb18G/CD147 in the radioresistance of human HCC cells. Deletion of CD147 significantly enhanced the radiosensitivity of HCC cells, and the antibody blockade of CD147 effectively decreased the tumor growth and metastatic potentials of HCC cells under irradiation in vivo.
HAb18G/CD147 is associated with intercellular interactions in tumor metastasis (28). Our previous studies have found that the HAb18G/CD147 extracellular domain interacts with the integrin β1 MIDAS motif to activate the downstream FAK/PI3K-Akt signaling pathway in HCC cells (24).

Integrins provide a transmembrane link for bidirectional signal transduction, namely outside-in and inside-out signaling (29–32). During outside-in signaling, the binding of extracellular ligands, for example, CD147, enhances separation of the cytoplasmic domains, allowing for their interaction with cytoskeletal ligands, for example, CD147, enhances separation of the cytoplasmic domains by intracellular ligands, for example, talin, would cause conformational changes of the integrin extracellular domains, thereby increasing the affinity of the integrin for extracellular ligands and enhancing outside-in signaling. In a recent study in our laboratory, we found that calpain contributes to the enhanced malignant properties of HCC cells, an effect that may be due to the cleavage of talin and the activation of integrin inside-out signaling (33, 34). In this study, CD147 deletion resulted in decreased levels of active integrin β1, calpain and cleaved talin, and suppressing calpain and talin expression resulted in dramatically reduced FAK phosphorylation. These results suggest a crucial role of CD147 in mediating integrin inside-out signaling.

FAK and PI3K/Akt play important roles downstream of the outside-in integrin signaling pathway. FAK signaling contributes to the proliferation and survival of cells and is thus linked to the cell adhesion-mediated radiosensitization of cancer cells (35). FAK can inhibit p53 expression (36, 37), and WT p53 protein has been proven to facilitate radiation-induced apoptosis (27). PI3K/Akt signaling is well characterized with respect to promoting cell survival and suppressing apoptosis. There is evidence that PI3K pathway inhibition enhances radiation-induced antiangiogenesis and increases tumor radiosensitivity by normalizing the tumor vasculature (38, 39). Our data showed that radiation activated FAK-PI3K/Akt signaling. Surviving radiosensitive HCC cells exhibit enhanced in situ tumor growth. These results indicate the association of radiation-enhanced growth capabilities with radiation-induced cell survival pathways in radiosensitive HCC cells. Treatment with an FAK or PI3K inhibitor promoted HCC cell apoptosis induced by radiation. Antibody-mediated targeting of integrin β1 also resulted in radiosensitization, suggesting the involvement of integrin outside-in signaling in the radiosensitivity of HCC cells. Thus, CD147 induces resistance to ionizing radiation not only by interacting with integrin β1 and activating the downstream FAK/PI3K-Akt pathway but also by mediating integrin β1 inside-out signaling and forming a positive feedback loop (Fig. 6F). The loss of WT p53 protein in SMMC-7721 cells may also contribute to the decreased radiosensitivity (Fig. 6F).

VEGF secretion by surviving tumor cells after sublethal ionizing radiation may decrease apoptosis, stimulate proliferation, and increase the angiogenic potential, all of which contribute to radiosensitivity (40–42). Postirradiation evaluations of VEGF/VEGFR2 may provide insights into the tumor response and can aid in planning the follow-up treatment (43). Radiation-enhanced activation of MMPs may promote tumor progression by decreasing apoptosis, stimulating proliferation, and increasing the angiogenic and invasive potentials (44, 45). We showed that ionizing radiation increased the levels of VEGF, MMP-2, and MMP-9, which enhanced the invasive and migratory potentials of HCC cells. The upregulation of VEGF, MMP-2, and MMP-9 are in part mediated by highly expressed CD147.

In summary, our data provide strong evidence for the contribution of CD147 to the radiosensitivity of HCC cells by mediating integrin β1 signaling. Inhibition of the HAb18G/CD147 integrin interaction may improve the efficiency of radiotherapy in HCC.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: J. Wu, J.-L. Jiang, Z.-N. Chen
Development of methodology: J. Wu, Y. Li, Y.-Z. Dang, Z.-N. Chen
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Wu, Y. Li, Y.-Z. Dang, H.-X. Gao
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Wu, Y. Li, Y.-Z. Dang, H.-X. Gao
Writing, review, and/or revision of the manuscript: J. Wu, Z.-N. Chen
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H.-X. Gao
Study supervision: Z.-N. Chen

Grant Support
This work was supported by grants from the National Natural Science Foundation of China (31371405: to J. L. Jiang; 31401222: to J. Wu; and 31401190: to Y. Li); the National S & T Major Project (2013ZX09301301: to Z.-N. Chen; 2012ZX100020017-002: to J. L. Jiang); the National High Technology Research and Development program of China 2012AA020302 (to J. L. Jiang) and Natural Science Foundation of Shaanxi Province 2014JM44121 (to Y. Li).

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Received August 11, 2014; revised November 20, 2014; accepted December 8, 2014; published OnlineFirst December 22, 2014.

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Molecular Cancer Therapeutics

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