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RESEARCH ARTICLE

Apicidin-resistant HA22T hepatocellular carcinoma cells massively promote pro-survival capability via IGF-IR/PI3K/Akt signaling pathway activation

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Abstract Despite rapid advances in the diagnostic and surgical procedures, hepatocellular carcinoma (HCC) remains one of the most difficult human malignancies to treat. This may be due to the chemoresistant behaviors of HCC. It is believed that acquired resistance could be overcome and improve the overall survival of HCC patients by understanding the mechanisms of chemoresistance in HCC. A stable HA22T cancer line, which is chronically resistant to a histone deacetylase inhibitor, was established. After comparing the molecular mechanism of apicidin-R HA22T cells to parental ones by Western blotting, cell cycle-regulated proteins did not change in apicidin-R cells, but apicidin-R cells were more proliferative and had higher tumor growth (wound-healing assay and nude mice xenograft model). Moreover, apicidin-R cells displayed increased levels of p-IGF-IR, p-PI3K, p-Akt, Bcl-xL, and Bcl-2 but also significantly inhibited the tumor suppressor PTEN protein and apoptotic pathways when compared to the parental strain. Therefore, the highly proliferative effect of apicidin-R HA22T cells was blocked by Akt knockdown. For all these findings, we believe that novel strategies to attenuate IGF-IR/PI3K/Akt signaling could overcome chemoresistance toward the improvement of overall survival of HCC patients.

Keywords Apicidin HA22T · Hepatocellular carcinoma cells · IGF-IR·PI3K · Akt

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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide in terms of numbers of cases (626,000 or 5.7 % of new cancer cases), but because of the very poor prognosis, the number of deaths is almost the same (598,000). Only a minority of HCCs is detected at an early enough stage for potentially curative therapies such as surgical resection or liver transplantation [1]. It is therefore the third most common cause of cancer death worldwide, causing an estimated 600,000 deaths in 2002 [2]. The areas of high incidence are sub-Saharan Africa, eastern and southeastern Asia, and Melanesia, with minor prevalence in Europe or America. However, current data indicate that its incidence is steadily increasing in the West [3]. The major risk factors for HCCs are infection with the hepatitis B and C viruses, both of which increase the risk of liver cancer some 20-fold [4]. Because hepatitis B virus (HBV) is more prevalent, the distribution of infection worldwide largely explains the patterns of liver cancer. HBV infection is highly prevalent in undeveloped countries and in some areas of Asia. There, the infection is acquired at birth or early in life, resulting in a very high incidence of HCC in children and adults [5, 6]. The individuals not eligible for curative therapy are generally treated with local ablative methods such as radiofrequency ablation or alcohol ablation if they have early disease, or with transarterial chemoembolization or radioembolization if they have intermediate–advanced disease [3]. There is currently no reliably effective therapy for patients with advanced or metastatic disease.

In HCC, multiple molecular alterations ensure the progressive growth of tumor cells. Rapid tumor growth is closely linked to chemotherapy resistance [7]. Chemoresistance is the major problem affecting HCC therapy; there is no effective chemotherapy for HCC because tumor cells develop resistance to cytotoxic drugs. Potentially curative therapies are offered to only one in every four patients coming to highly committed centers [5]. *p53* function is frequently altered in HCC as it is in many other solid tumors and contributes to tumor growth and chemoresistance [8]. Therefore, transferring a wild-type *p53* gene to *p53*-negative HCC cells inhibits its growth and increases its sensitivity to chemotherapy [9]. The solution of HCC chemoresistance is now the most important issue today.

Histone acetylation and deacetylation play critical roles in modulating chromatin topology and gene transcription [10]. Deacetylation is a process mediated by histone deacetylase (HDAC), which removes the acetyl group from the histone tails, resulting in histones to wrap more tightly around the DNA and interfere with gene transcriptions. HDACs are usually overexpressed in tumors compared in surrounding normal tissue and highly correlated with poor prognosis and survival rate [11]. HDACs are implicated in gene regulations, stress resistance, and tumorigenesis in HCC [12]. Therefore, HDAC inhibitors (HDACi) are a novel class of anticancer treatments that can inhibit proliferation and induce differentiation and/or apoptosis of tumor cells, with slight toxicity to normal cells [13, 14]. The currently accepted rationale for their antitumor efficacy is suppression of tumor cell proliferation, metastasis, angiogenesis, invasion, and survival with slight or no toxicity to normal cells. Therefore, inhibition of histone deacetylases results in upregulation of proapoptotic proteins together with downregulation of anti-apoptotic proteins which potentially represents an efficient tool to lead cell death.

Apicidin is a novel HDAC inhibitor derived from a fungal metabolite [15–17]. Apicidin has been reported with a potent broad spectrum of antiproliferative activity against various cancer cell lines [18–20]. The combination of apicidin with doxorubicin enhances the antitumor effects of doxorubicin on caspase activation and tumor growth in HCCs [21]. However, the growth-inhibitory concentrations of apicidin in HCCs were higher than the other cancer cell lines (Table 1). Therefore, the induction of side effects and chemoresistance by apicidin could be expected in HCC treatment.

The type I insulin-like growth factor receptor (IGF-IR) and its ligand play a critical role in the regulation of cellular proliferation and apoptosis [22]. The upregulated expression of IGF-IR and IGF-I has been documented in many animal and human malignancies [23]. Tumor cells with functional IGF-IR may be able to increase their own growth by synthesis of endogenous IGFs. In addition, clinical studies identified high plasma IGF-I levels as a potential risk factor for carcinomas [24]. Ligand binding to IGF-IR can trigger multiple signaling pathways such as the PI3-kinase/Akt pathway implicated in the transmission of cell survival signals [24, 25]. In vivo inhibition of IGF-IR or IGF-I expression by antisense RNA or IGF-I peptide analogs caused a suppression of tumor cell growth [24, 26], identifying this receptor as a target for cancer therapy [3]. In previous study, the phosphorylation levels of IGF-IR were higher in the chemoresistant cancer cells than in parental ones [27]. However, inhibition of IGF-IR would restore chemosensitivity in the chemoresistant cell

Table 1 Growth-inhibitory concentrations of apicidin in various cell lines

Cell lines	Cell type	IC_{50} (μM)	Reference
MCF7	Human breast cancer cell line	1.88	[18]
MCF10	Human breast cancer cell line	0.19	[19]
AGS	Human gastric adenocarcinoma cell line	0.21	[18]
HepG2	Human hepatoma cell line	0.5	[20]
Hep3B	Human hepatoma cell line	3	[21]
Huh7	Human hepatoma cell line	4	[21]
HA22T	Human hepatoma cell line	5	–
CCD-18Co	Human normal colon cell line	3.78	[18]

lines [27–30]. IGF-IR makes a significant contribution to the resistance to clinical therapy [31]. Therefore, IGF-IR inhibition may prove to be the therapeutic strategy offered to patients with otherwise untreatable disease. Here, we aim to investigate if apicidin-resistant HA22T hepatocellular carcinoma cells highly promote cell survival capability and to reveal the roles of IGF-IR/PI3K/Akt signaling pathway.

Materials and methods

Cell culture HA22T cells were maintained in Dulbecco's minimum essential medium (Sigma, MO) containing 10 % charcoal-treated Characterized Fetal Bovine Serum (HyClone, UT) and 1 % penicillin (Invitrogen, CA).

Development of apicidin-resistant cell lines To establish stable liver cancer cell lines chronically resistant to apicidin, HA22T cells were exposed to increasing concentrations of apicidin. HA22T cells were first exposed to 5 μM of apicidin, which resulted in greater than 95 % cell death. Once surviving cells reached to 80 % confluency, they were passaged twice in this same concentration of apicidin, after which the process was repeated at gradational doses of apicidin until a cell population was selected that demonstrated at least a 3-fold greater IC50 to apicidin than the parental cell lines.

Akt small interfering RNA (siRNA) transfection Apicidin-R HA22T cells were seeded at a density of $2–3\times10^5$ cells in 60-mm culture dishes. Transient transfections were carried out by the proprietary cationic polymer reagent (TurboFect™ in vitro Transfection Reagent; Fermentas, MA) following the manufacturer's instruction. Twenty-five nanomolars of double-stranded siRNA sequences targeting Akt mRNAs was obtained from Santa Cruz Biotechnology. The nonspecific siRNA (scramble) consisted of a nontargeting sequence. Specific silencing was confirmed by immunoblotting with cellular extracts after transfection.

Whole cell extract The cells were extracted in a cell lysis buffer (50 mM Tris base, 0.5 M NaCl, 1.0 mM EDTA, 1 % NP-40, 1 % glycerol, 1 mM β-mercaptoethanol, and proteinase K inhibitor). The extracts were clarified by centrifugation.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide) assay The cell viability after apicidin treatment was measured using MTT assay (Sigma, MO). Cells were plated in triplicate in 24-well plates and treated with increasing concentrations of apicidin. After 48 h of incubation, 0.5 mg/mL of MTT was added to each well for an additional 4 h. The blue MTT formazan precipitate was then dissolved in 100 μL DMSO. The absorbance at 570 nm was measured on a multi-well plate reader. Cell viability was expressed as a percentage of control.

Trypan blue exclusion: growth and cell viability test To evaluate growth and viability of the treated and untreated cells, the percentage of viable–nonviable cell cytotoxic potential of 4′,5′,7-trihydroxyisoflavone was determined, using trypan blue exclusivity stain. Cell growth and viability was measured by adding 0.4 % trypan blue in 0.9 % saline to a 50 % dilution, and cells were counted using the hemocytometer according to the standard procedure. Briefly, 0.5 mL of the trypan blue solution was transferred to a test tube, and 0.3 mL of PBS plus 0.2 mL of the trypsinized cell suspension (dilution factor of 5) were added. The final solution was thoroughly and gently mixed and allowed to stand for 5 min. Then a drop of this dye–cell suspension was loaded onto both chambers of the hemocytometer. Cells were examined and counted in duplicates under light microscope at ×200 (Olympus BH2). The percentage of cell viability was calculated by the following formula: cell viability (x,)=no. of viable cells (unstained cells)×l00/total no. of cells (stained and unstained). Thus, the percentage of viable and nonviable cells (spontaneous and treatment-induced death) was determined, and the intertreatment results were compared with the nontreatment control.

Lowry protein assay Add water to the tube with the protein standard (2 mg/mL bovine serum albumin (BSA)) for dilution to make a 0.5-mg/mL BSA solution. For a standard curve, prepare a range of protein standards (0, 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL) with the diluted BSA (0.5 mg/mL). Protein was first reacted with alkaline cupric sulfate in the presence of tartrate (2 % Na-K tartrate: 1 % $CuSO{\cdot}4.5H_2O$: 2 % Na_2CO_3 in 0.1 N NaOH=1:1:98) during a 10-min incubation at room temperature. During this incubation, a tetradentate copper complex forms from four peptide bonds and one atom of copper. Following the incubation, Folin–Ciocalteu's phenol reagent (Sigma-Aldrich, MO) was added. It is believed that the color enhancement occurs when the tetradentate copper complex transfers electrons to the phosphomolybdic/phosphotungstic acid complex. After 30 min of incubation at room temperature, OD at value 750 nm was dictated by the ELISA reader.

Western blotting Cultured cells were lysed with lysis buffer (250 mM sucrose, 50 mM Tris–HCl, 5 mM imidazole, 2.5 mM EDTA, 2.5 mM DTT, and 0.1 % Triton X-100, at pH 7.4), and protein concentration was measured using the Lowry protein assay. Briefly, an aliquot of each sample equivalent to 30 μg of protein was boiled after the addition of the appropriate amount of 5× sample buffer (5 mM EDTA, 162 mM DTT, 5 % SDS, 50 % glycerol, 0.5 L bromophenol blue, and 188 mM Tris, at pH 8.8). The samples were separated on 10 % SDS–polyacrylamide gels and electrophoretically transferred onto nitrocellulose filters using the Bio-Rad

electrotransfer system (Bio-Rad Laboratories, Munich, Germany). Equal transfer was verified by Ponceau S staining of the membranes. Antigen–antibody complexes were visualized with HRP-coupled secondary antibodies (goat anti-mouse and goat anti-rabbit) (Santa Cruz Biotechnology, CA) and a custom-made ECL detection system (2.5 mM luminol, 0.4 mM *para*-coumaric acid, 10 mM Tris base, and 0.15 L H_2O_2, at pH 8.5). The following antibodies used were purchased from Santa Cruz Biotechnology (Santa Cruz, CA): β-actin (C4), Bad, Bcl-xL (H-5), cyclin A (H-432), cyclin B1 (H-433), cyclin D1 (HD11), cytochrome c, FADD, Fas, FasL, c-Myc (C-33), PCNA (FL-261), PEA3 (G-10), p-PI3K (Tyr 508) p85α, and α-tubulin (B-7). Antibodies against p-Akt (Ser473), p-Bad (Ser136), PTEN (138G6), p-IGF-IR (Y1161), and Bcl-2 were purchased from Cell Signaling Technology (Beverly, MA).

Wound healing assay Cells were initially seeded uniformly onto 60-mm culture plates with an artificial "wound" carefully created at 0 h, using a P-200 pipette tip to scratch on the subconfluent cell monolayer. After 48 h of culture in 1 % serum-supplemented DMEM, the cell migration was observed by an eyepiece of a phase contrast microscope.

Liver cancer tumor model NU/NU nude mice were divided into two groups with each group containing six animals. In group I, HA22T cells (1×10^6 in 100 μL DMEM) were subcutaneously injected into the left flank of NU/NU mice as a control. Group II was injected with apicidin-R HA22T cells (1×10^6).

Tumor volumes were measured on 1, 2, 3, and 4 weeks with a caliper and calculated according to the formula $[(L\times W^2)/2]$, where L and W stand for length and width, respectively [33]. All xenografted mice were sacrificed after tumor inoculation, and the tumors were excised and weighted.

Statistical analysis Each sample was analyzed based on results that were repeated at least three times, and SigmaPlot 10.0 software and standard t test were used to analyze each numeric data. In all cases, differences at $P<0.05$ were regarded as statistically significant; the ones at $P<0.01$ or $P<0.001$ were considered at higher statistical significances.

Results

Determination of the IC50 concentration of apicidin in HA22T cells HA22T cells was treated with different concentrations of apicidin (0, 2.5, 5, 10, and 15 μM) by MTT assay, and cell viability was measured after 48 h. We have observed that apicidin can inhibit cell viability in a dose-dependent manner in HA22T cells (Fig. 1).

Development of apicidin-resistant cell lines To establish stable liver cancer cell lines chronically resistant to apicidin, HA22T cells were exposed to increasing concentrations of apicidin. HA22T cells were first exposed to 5 μM of apicidin, which resulted in greater than 95 % cell death. Once surviving cells reached 80 % confluence, they were passaged twice in this same concentration of apicidin, after which the process was repeated at gradational doses of apicidin until a cell population was selected that demonstrated at least a 3-fold greater IC50 to apicidin than the parental cell lines.

There was no change of cell cycle-regulated proteins of apicidin-resistant cells The PCNA, c-Myc, cyclin A, cyclin

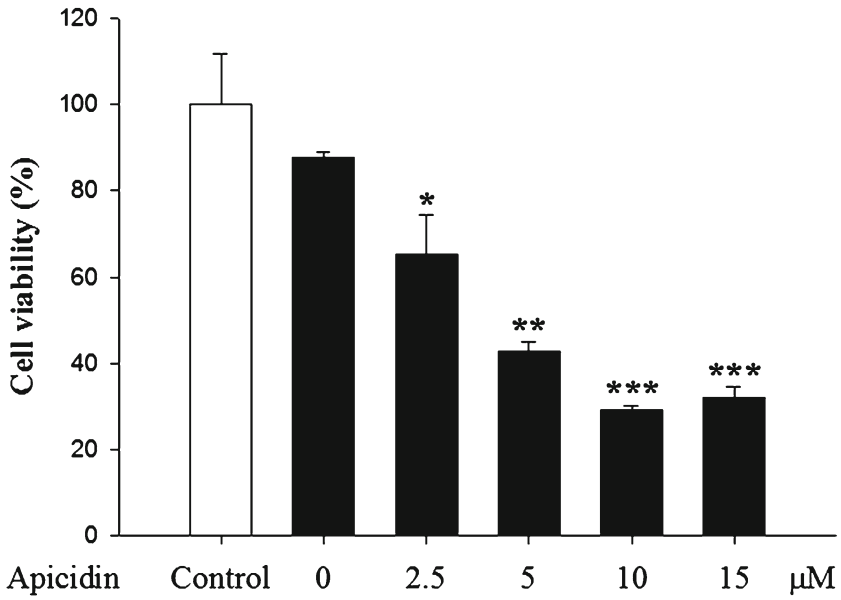

Fig. 1 Determination of IC50 concentration of apicidin to the HA22T cells. Cell viability assay of apicidin (0, 2.5, 5, 10, and 15 μM) on HA22T cells after 48-h treatments. Data are expressed as mean ± SD, $n=3$. *$P<0.05$; **$P<0.01$; and ***$P<0.001$ represent significant differences when compared with the control group

D, and cyclin B1 protein levels were not different between the HA22T and apicidin-resistant HA22T cells (Fig. 2).

Apicidin-R cells are more proliferous when compared to the parental HA22T cells in vitro and in vivo To determine whether the apicidin-R HA22T cells were more proliferous than the parental HA22T cells, the cell proliferation was tested using the wound-healing assay. The results showed that the apicidin-R HA22T cells enhanced proliferously when compared to the HA22T cells (Fig. 3a, b). The growth rate of tumor tissues in HA22T and apicidin-R HA22T xenografted nude mice ($n=6$, individually) was compared. Tumor volume was measured after the injection of HA22T (1×10^6) or apicidin-R HA22T cells (1×10^6) into the left flank of NU/NU mice. The tumor growth in apicidin-R HA22T cell xenograft mice is significantly higher than that in HA22T cell xenograft mice from 1 to 4 weeks (Fig. 3c).

IGF-IR/PI3K/Akt pathway was highly activated in apicidin-R HA22T cells To determine whether the survival pathway was involved in apicidin-R HA22T cells, we assessed the levels of the IGF-IR/PI3K/Akt pathway at protein levels by Western blotting. Apicidin-R HA22T cells displayed increased levels of p-IGF-IR, p-PI3K, p-Akt, Bcl-xL, and Bcl-2 when compared to the parental HA22T cells (Fig. 4). Moreover, the tumor suppressor PTEN protein level was significantly inhibited in apicidin-R HA22T cells (Fig. 4).

The intrinsic and extrinsic apoptosis pathways were both highly suppressed in apicidin-R HA22T cells The results showed that cytochrome C and Bad were significantly suppressed in apicidin-R HA22T cells (Fig. 5a). In addition, apicidin-R HA22T cells had more inhibition of FAS/L and FADD when compared to the parental HA22T cells (Fig. 5b).

Knockdown of Akt by gene silencer could inhibit extraordinary proliferation effect of apicidin-R HA22T cell Apicidin-R HA22T cells were transfected with Akt siRNA for 24 h. The cell number was measured after 24, 48, and 72 h by trypan blue exclusion. Apicidin-R HA22T cells displayed significant cellular proliferation than HA22T parental cells from 24 to 72 h. However, the extraordinary apicidin-R HA22T cell proliferative effect was totally reversed to the HA22T parental cell level by a 25-nM Akt siRNA transfection (Fig. 6).

Discussion

Despite rapid advances in the diagnostic and surgical procedures, HCC remains one of the most difficult human

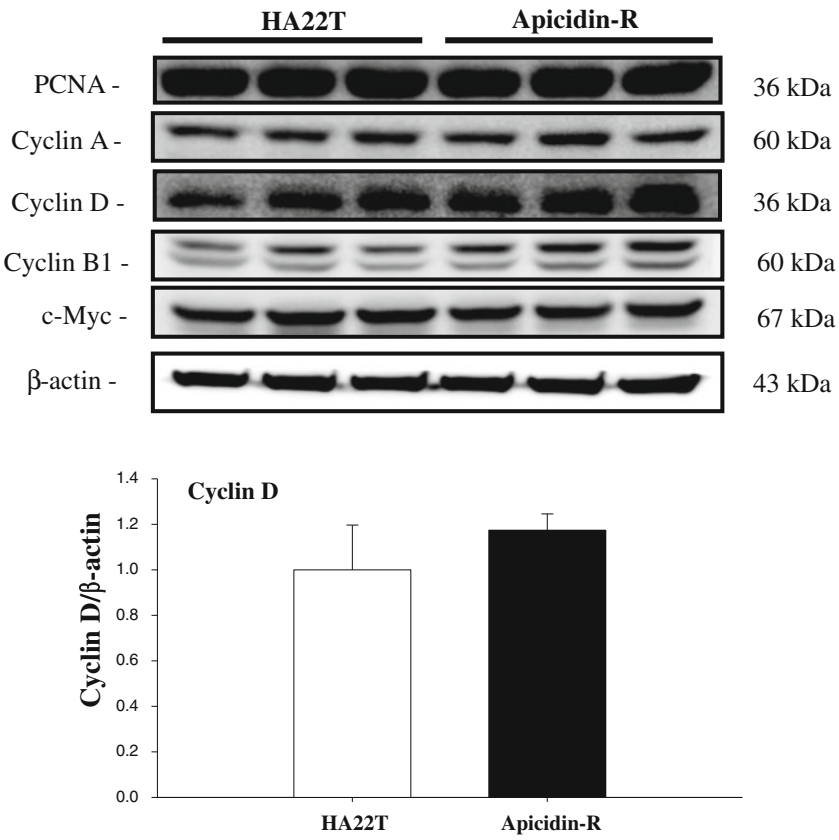

Fig. 2 There was no change of cell cycle-regulated proteins of apicidin-resistant cells. Western blot analysis of total cellular lysates using PCNA, c-Myc, cyclin D, cyclin B1, and β-actin antibodies in HA22T and apicidin-resistant (*apicidin-R*) cells. For cyclin D, values were quantified as fold of apicidin-R relative to the parental HA22T cells levels. There was no statistical effect compared with the HA22T cells

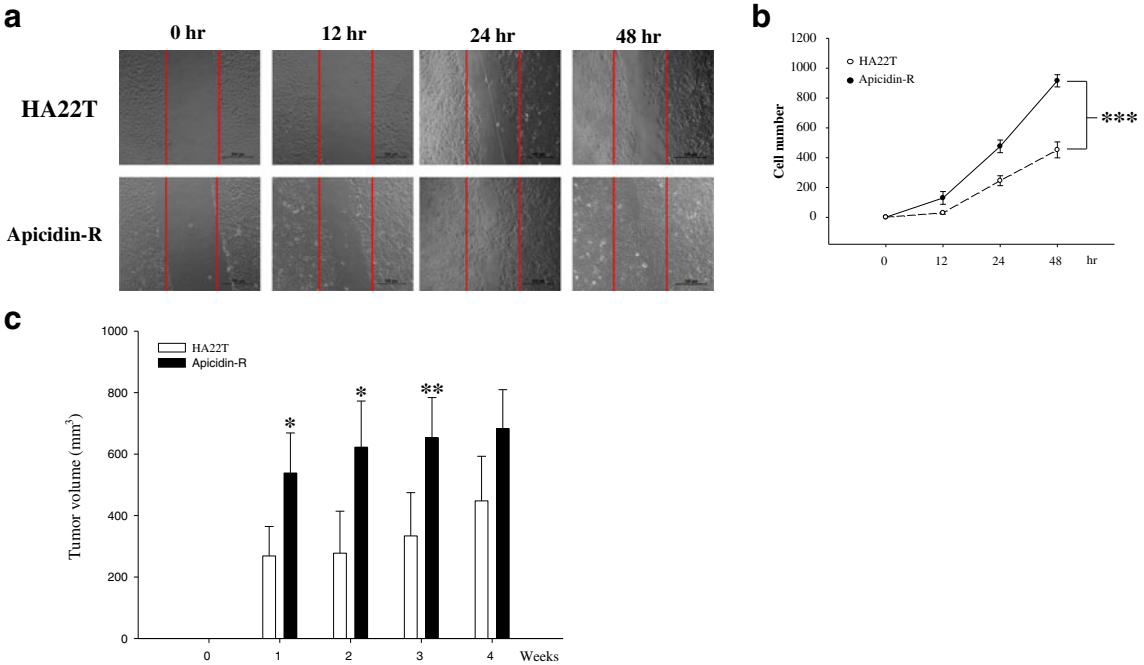

Fig. 3 Apicidin-R cells were more proliferous when compared to the HA22T cells. **a** and **b** Wound healing assay was conducted when HA22T and apicidin-R cells were confluent and cell motility were observed after 12, 24 and 48 h of incubation microscopically. **c** Comparison of the growth rate of HA22T and apicidin-R HA22T xenograft tumor tissues in nude mice ($n=6$, individually). Tumor volume was measured after injection of HA22T (1×10^6) or apicidin-R HA22T cells (1×10^6) into the left flank of NU/NU mice. *$P<0.05$; **$P<0.01$; and ***$P<0.001$, statistically significant compared with the parental HA22T cells

malignancies to treat. This may be due to the chemoresistant behaviors of HCC. In addition, there has been no systemic therapy proven effective in improving the overall survival of patients diagnosed with this deadly disease [33]. It is believed that acquired resistance to therapy could be overcome by understanding the mechanisms by which HCC becomes chemoresistant. Thus, it will improve the overall survival of HCC patients. In this study, we have used the parental HA22T cells and apicidin-resistant HA22T cells to investigate the molecular mechanism of resistance and associated cellular behaviors. We successfully established the stable HA22T cancer cell lines, which are chronically resistant to apicidin, and determined the IC50 concentration of apicidin in HA22T cells (Fig. 1).

After comparing the molecular mechanism of apicidin-R HA22T cells to parental HA22T cells, there were no cell cycle-regulated proteins changed in apicidin-R HA22T cells (Fig. 2), but more proliferation of apicidin-R HA22T cells was observed by the wound-healing assay (Fig. 3a, b). Moreover, as with comparing the growth rate of tumor tissues in HA22T and apicidin-R HA22T xenografted nude mice, the tumor growth in apicidin-R HA22T cell xenograft mice is significantly higher than that in HA22T cell xenograft mice from 1 to 4 weeks (Fig. 3c).

p53 function highly correlates to tumor growth and chemoresistance [8]. The HA22T cell line had abnormal expression of the *p53* gene, as well as no *p53* protein was detectable [34]. As expected, there were no detectable *p53* proteins in the parental HA22T and apicidin-R HA22T cells (data not shown). IGF-IR can trigger multiple signaling pathways such as the PI3-kinase/Akt pathway implicated in the transmission of cell survival signals [25]. Inhibition of IGF-IR would restore chemosensitivity in the chemoresistant cell cancer lines [27, 31]. Apicidin-R HA22T cells did not only display increased levels of p-IGF-IR, p-PI3K, p-Akt, Bcl-xL, and Bcl-2 but also significantly inhibited the tumor suppressor PTEN protein level when compared to the parental HA22T cells (Fig. 4). In addition, apicidin-R HA22T cells displayed significant cellular proliferation than the HA22T parental cells from 24 to 72 h. However, this extraordinary apicidin-R HA22T cell proliferative effect was totally reversed to the HA22T parental cell level by a 25-nM Akt siRNA transfection (Fig. 6). Evidences have shown that IGF-IR acts as an anti-apoptosis agent by enhancing cell survival [35]. These changes in IGF-IR/PI3K/Akt pathway suggested that apicidin-R HA22T cells have implicated in the transmission of cell survival signals.

Mitochondrial membrane permeabilization was regulated by an elegant balance of opposing actions of proapoptotic and

anti-apoptotic Bcl-2 family members. Mitochondrial functions are suspected as well to contribute to the development and progression of cancer and to resistance to therapy [32, 36–41]. Apicidin-R HA22T cells were significantly suppressed cytochrome c and Bad (Fig. 5a). Bad promoted the release of cytochrome c through the formation of transmembrane channels across the mitochondrial outer membrane [42–45]. Meanwhile, Bcl-2 and Bcl-xL could delay cytochrome c

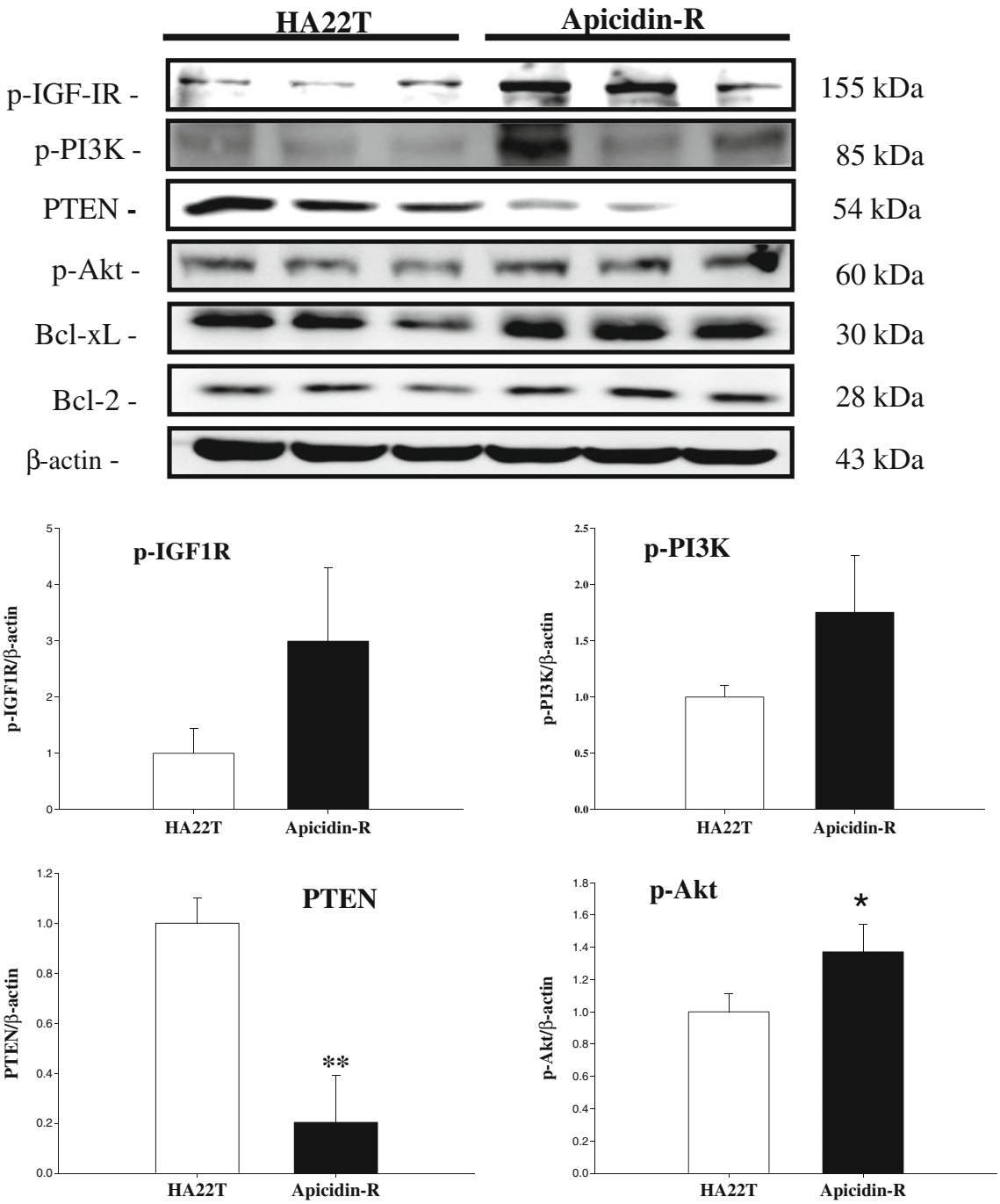

Fig. 4 The PI3K/Akt pathway is highly activated in apicidin-R cells. The Western blots were carried out with anti-p-IGF-IR, p-PI3K, PTEN, p-Akt, Bcl-xL, Bcl-2, and β-actin antibodies. The expression levels were qualified by calculating the relative expression level of β-actin (as the internal control) and are shown as the mean expression levels from three independents; *columns*, mean ($n=3$); *bars*, SD. *$P<0.05$, statistically significant compared with the parental HA22T cells

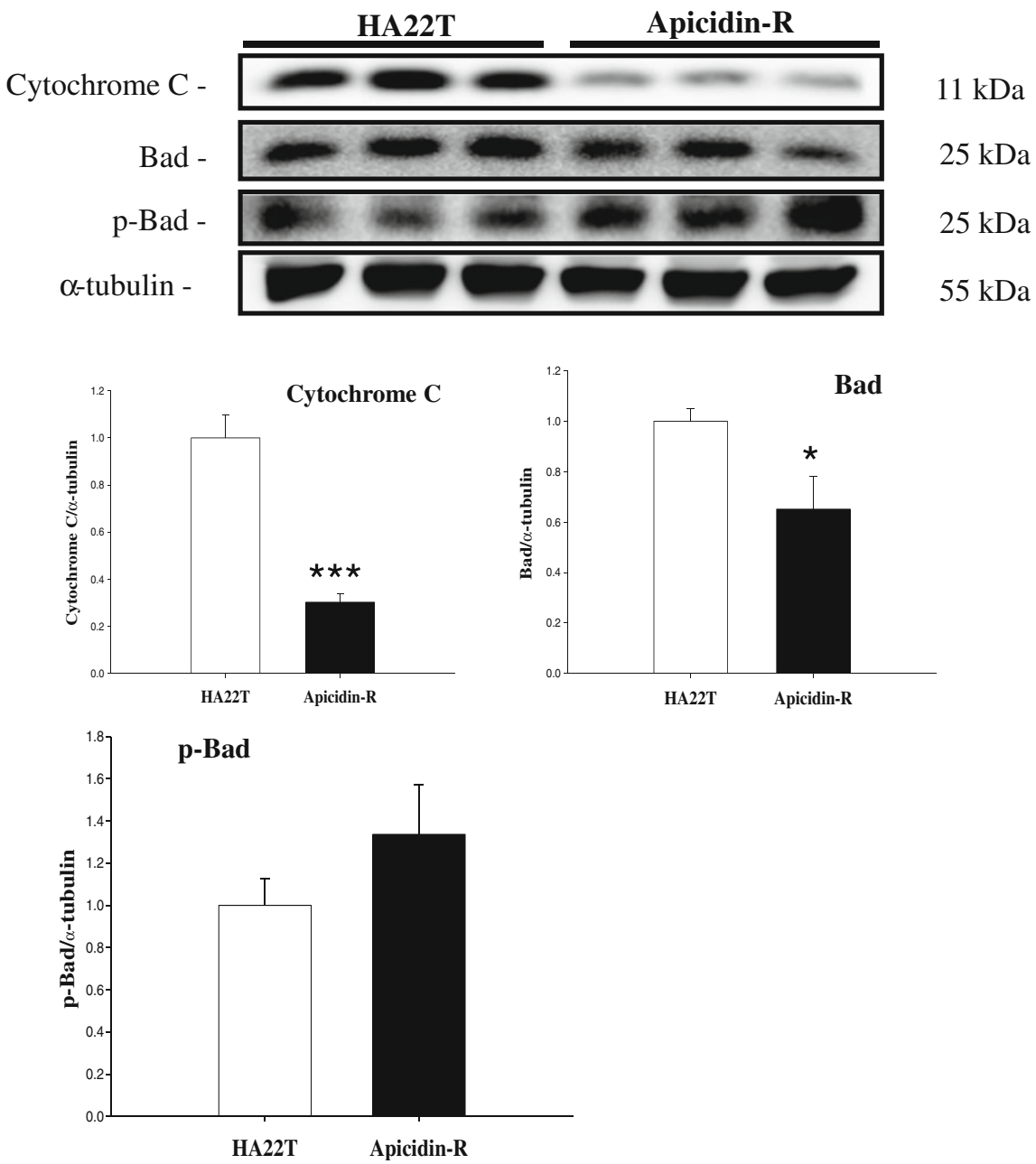

Fig. 5 Apicidin-R cells significantly suppressed both the intrinsic and extrinsic apoptosis pathways. **a** Western blot analyzed of cytochrome c, Bad, and p-Bad in HA22T and apicidin-R cells. *$P<0.05$, values were quantified as fold of apicidin-R relative to the parental HA22T cells levels. **b** The levels of FAS, FAS/L, FADD, and β-actin were examined by Western blot. *$P<0.05$, compared with the parental HA22T cells

release and abort the apoptotic response, leading to cell survival [42, 46, 47]. The release of cytochrome c to the cytosol is considered among the major steps in the intrinsic death pathway [48–50]. In addition, vertebrates have evolved an additional route to cell death that is triggered by cell surface receptors leading to activation of pro-caspase-8. In the death receptors of the TNF receptor family (such as Fas), by their cognate ligands, the aggregated receptors recruit the adaptor protein FADD promoting cell death [51, 52]. Apicidin-R HA22T cells had more inhibition of FAS/L and FADD when compared to the parental HA22T cells (Fig. 5b). The ability of tumor cells to escape apoptosis plays a key role in promoting resistance to conventional chemotherapy [53–55]. These results indicated that apicidin-R HA22T cells inhibited not only intrinsic apoptosis pathway but also extrinsic apoptosis pathway which promoted resistance to conventional chemotherapy.

In summary, we have shown that apicidin-R HA22T cells greatly enhance cell survival effect via massively

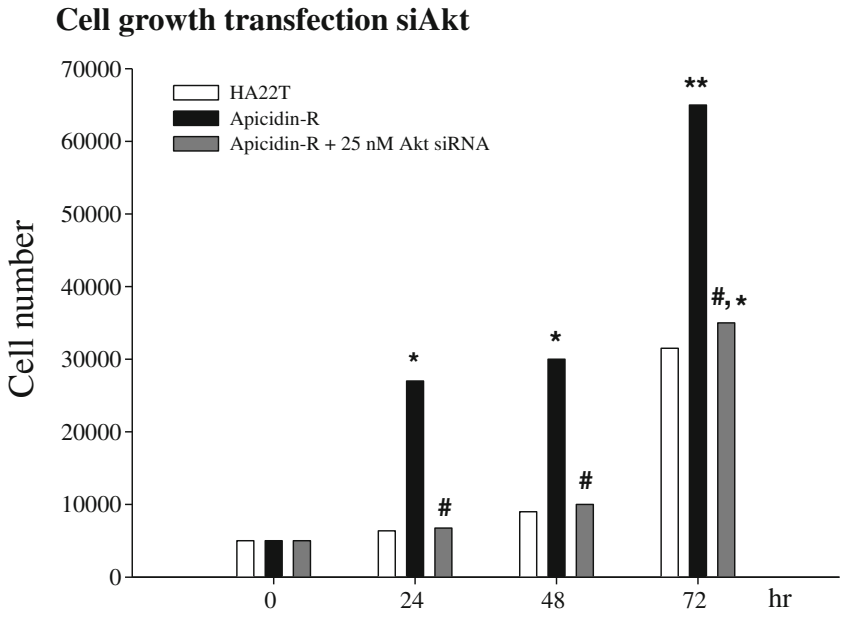

Fig. 6 Downregulation of Akt could greatly inhibit apicidin-R HA22T cell proliferation. Apicidin-R HA22T cells were transfected with Akt siRNA for 24 h. The cell number was measured after 24, 48, and 72 h by trypan blue exclusion. *$P<0.05$; **$P<0.01$, statistically significant compared with the parental HA22T cells. #$P<0.05$, statistically significant compared with the apicidin-R HA22T cells

promoting IGF-IR/PI3K/Akt signaling pathway activation. Moreover, promoting Bcl-2 and Bcl-xL delays cytochrome c release and aborts the apoptotic response, leading to cell resistance to conventional chemotherapy survival. But the downregulation of Akt could totally abolish apicidin-R HA22T cell extraordinary proliferation. Therefore, we believe that novel strategies by IGF-IR/PI3K/Akt signaling downregulation could overcome chemoresistance toward the improvement of overall survival of HCC patients (Fig. 7).

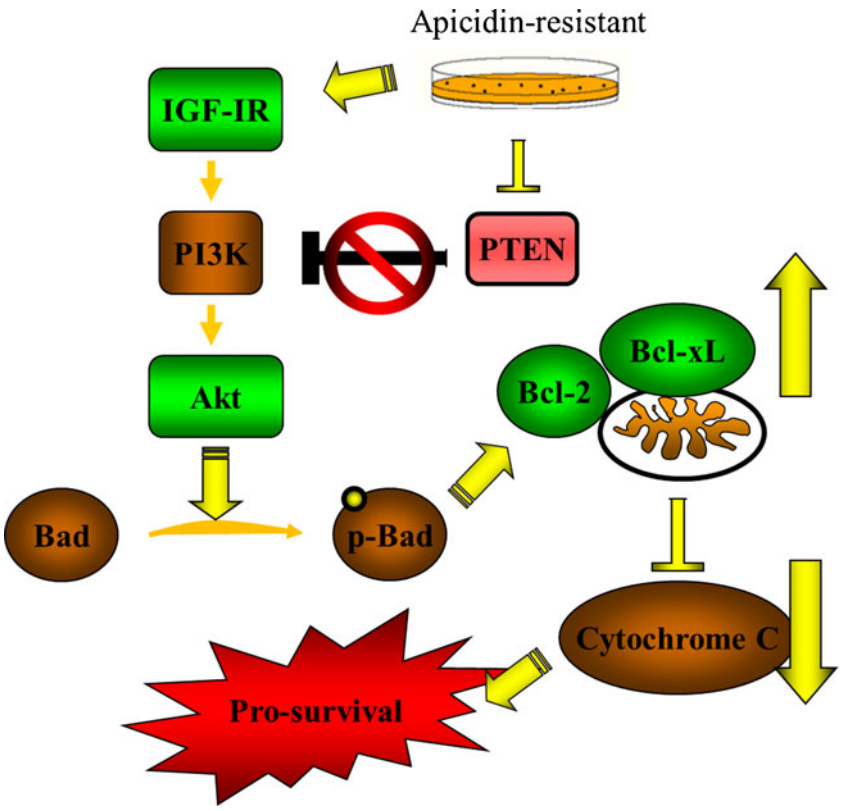

Fig. 7 Apicidin-R cells enhance pro-survival capability via IGF-IR/PI3K/Akt signaling pathway activation

Conflicts of interest None

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