High doses of tyrosine kinase inhibitor PTK787 enhance the efficacy of ischemic hypoxia for the treatment of hepatocellular carcinoma: dual effects on cancer cell and angiogenesis

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Abstract

The present study aimed to investigate the therapeutic efficacy of combining vascular endothelial growth factor (VEGF) receptor blockade using tyrosine kinase inhibitor PTK787 with hypoxia for the treatment of hepatocellular carcinoma (HCC). The in vivo effects of the treatments were determined in a rat orthotopic HCC model, in which hypoxia was generated by hepatic artery ligation (HAL). Compared with HAL alone, PTK787 combined with HAL significantly prolonged the animal survival, reduced the tumor size, induced more tumor tissue necrosis and apoptosis, and down-regulated the expression of von Willebrand factor. The mechanism was explored in vitro using murine HCC and endothelial cell lines, respectively. PTK787 combined with hypoxia decreased the expression of VEGF and VEGF receptors in both cell lines and suppressed the cell viability by induction of cell cycle arrest and promotion of apoptosis. Up-regulation of cleaved form caspase-9 and down-regulation of Bcl-2 and cyclin D1 were detected with the combined treatment. Hypoxia sensitized endothelial cells to the inhibitory effect of PTK787 on forming tubular-like structure. The motility of tumor cells was inhibited by hypoxia and the combined approach, with down-regulation of Rac1, Rho, and phosphorylated Akt expression. However, in the endothelial cells, the combined treatment inhibited the hypoxia-enhanced cell motility, with suppressed Rac1, Rho, and phosphorylated Akt expression. In conclusion, PTK787 combined with hypoxia achieved a better therapeutic efficacy than hypoxia alone through enhancing hypoxia-induced antitumor cell effect and preventing the activation of angiogenic process. [Mol Cancer Ther 2006;5(9):2261–70]

Introduction

Transcatheter arterial chemoembolization, which combines the effects of hypoxia and chemocytotoxicity, is a widely used treatment for unresectable hepatocellular carcinoma (HCC; refs. 1–3). However, the long-term survival of patients remains unsatisfactory (4, 5). HCC is a hypervascularized solid tumor, and oxygen depletion that is induced by the blockade of hepatic artery blood supply could arrest tumor cell proliferation and lead to apoptosis and necrosis in the tumor tissue (6–9). However, objective tumor response rate to transcatheter arterial chemoembolization is only about 35% to 40% (1, 2, 4, 5). Hypoxia-mediated angiogenesis has been postulated as one of the major factors that result in the treatment failure of this therapeutic approach (10, 11).

The hypoxia-mediated angiogenesis is a multifactorial process, in which vascular endothelial growth factor (VEGF) plays a pivotal role (12–15). Our previous study in an animal model, which mimicked the clinical setting of transcatheter arterial chemoembolization for HCC, showed that hepatic artery ligation (HAL) generated a hypoxic condition in the tumor tissue and stimulated the expression of VEGF in the tumor-bearing rats, suggesting the potential role of VEGF in mediating post-treatment angiogenesis (16). Therefore, we hypothesize that blockade of VEGF pathway may enhance the therapeutic efficacy of hypoxia.

VEGF mediates its proangiogenic and prosurvival functions mainly through activation of its receptors [VEGF receptor (VEGFR)], fms-like tyrosine kinase-1 (Flt-1) and fetal liver kinase-1 (Flk-1), and subsequent downstream molecules (17, 18). The VEGF-VEGFR interaction initiates the up-regulation of several cell survival–related molecules, such as Akt and phosphatidylinositol 3-kinase, which can prevent both endothelial and tumor cells from undergoing apoptosis (19, 20). In addition, activation of the VEGF-VEGFR pathway also augments the tube formation and migration activity of endothelial cells, resulting in enhanced neovascularization (21, 22).

Based on the importance of VEGF-VEGFR pathways in angiogenesis and cell survival, several approaches have been attempted to achieve anticaner therapy by blockade of VEGF or VEGFRs in both animal and clinical settings (23–25). Among the therapeutic approaches targeting
VEGFRs, PTK787, a tyrosine kinase inhibitor currently in clinical trials for anticancer therapy, exhibits potent anti-VEGFR effects in a variety of tumors, including our finding in HCC (26–29). As our previous studies showed that VEGF and VEGFRs were highly expressed in HCC and the expression of VEGF was also up-regulated in the tumor tissues after ischemic hypoxia (16, 30, 31), we did the present study to investigate the potential therapeutic efficacy of combining VEGFR blockade with hypoxia for the treatment of HCC in the rat orthotopic HCC model that we have described previously.

Materials and Methods

Orthotopic HCC Model in Rat Liver

Adult male Buffalo rats, weighing 250 to 300 g, were purchased from the Charles River Laboratories (Wilmington, MA). They were maintained under standard conditions and cared for according to the institutional guidelines for animal care. All the animal experiments were approved by the Committee on the Use of Live Animals in Teaching and Research at the University of Hong Kong (Hong Kong, China). McA RH7777 rat HCC cell line (CRL-1601) was purchased from the American Type Culture Collection (Manassas, VA). A total of 1 × 10⁶ cells were inoculated into the left lobe of the liver. Fourteen days after tumor cell inoculation, the animals were randomly divided into the left lobe of the liver. Fourteen days after tumor cell inoculation, the animals were randomly divided into the following four experimental groups: (a) sham operation (n = 7); (b) PTK787 (50 mg/kg oral feeding daily; n = 6; Novartis Pharmaceuticals Ltd., Basel, Switzerland); (c) HAL (n = 6); and (d) HAL combined with PTK787 (HAL+PTK787; n = 7). Another 48 rats (4 rats at each time point in each group) were sacrificed on days 0, 2, and 14 after treatment for the measurement of tumor size and collection of tissues.

Histologic Study

When the animals were killed, tumor tissues were obtained, fixed in 10% buffered formalin, and embedded in paraffin for the determination of histology by H&E staining, detection of apoptotic cells by terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (Roche, Basel, Switzerland), and detection of von Willebrand factor expression, a marker for angiogenesis/neo-vascularization, using Blood Vessel Staining kit (Chemicon, Temecula, CA).

In vitro Study

Cell Lines. The rat HCC cell line and a murine endothelial cell line (CRL-2279, American Type Culture Collection) were maintained as monolayer culture in DMEM with 10% fetal bovine serum and 1% penicillin (Life Technologies, China). McA RH7777 rat HCC cell line (CRL-1601) was purchased from the American Type Culture Collection (Manassas, VA). A total of 1 × 10⁶ cells were inoculated into the left lobe of the liver. Fourteen days after tumor cell inoculation, the animals were randomly divided into the following four experimental groups: (a) sham operation (n = 7); (b) PTK787 (50 mg/kg oral feeding daily; n = 6; Novartis Pharmaceuticals Ltd., Basel, Switzerland); (c) HAL (n = 6); and (d) HAL combined with PTK787 (HAL+PTK787; n = 7). Another 48 rats (4 rats at each time point in each group) were sacrificed on days 0, 2, and 14 after treatment for the measurement of tumor size and collection of tissues.

Cell viability and proliferation was determined by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Cell viability and proliferation was determined by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Both HCC and endothelial cells (1 × 10⁴) were inoculated into each well of a 96-well plate and treated with 10% fetal bovine serum–DMEM containing different doses of PTK787 (0, 10, 20, and 40 µmol/L, respectively) under normoxia (20% O₂) or hypoxia (0.1% O₂) for 12 hours. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was then added into each well and the cells were incubated for another 4 hours. The reaction was stopped with 0.04 mol/L hydrochloric acid (isopropanol) and measured at absorbance of 570 to 630 nm in a Vₘₐₓ kinetic microplate reader (Molecular Devices Corp., Sunnyvale, CA). The cell proliferation index was expressed as mean ± SD.

Cell Cycle Analysis. Cells were harvested after treatment and fixed in absolute ethanol at 4°C for 12 hours. After washing with 1 × PBS twice, the cells were stained with propidium iodide at 37°C for 30 minutes before proceeding to a FACSCalibur (Becton Dickinson Immunocytometry Systems, San Jose, CA).

Cytofluorometric Apoptosis Analysis. The HCC and endothelial cells were harvested after different treatments, labeled with Annexin V-FITC antibody (BD Biosciences PharMingen, San Diego, CA) at room temperature for 15 minutes, and detected in a FACSCalibur. Unstained cells were used as a negative control.

Tube Formation Assay. The tube formation ability of the endothelial cells was determined by the In vitro Angiogenesis Assay kit (Chemicon). After coating the bottom of a 96-well plate with a solid gel of basement proteins, 5 × 10⁴ endothelial cells were inoculated on the surface of the gel, incubated with different concentrations of PTK787 under normoxic or hypoxic conditions for 6 hours before staining with 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, and assessed under a microscope.

Migration Assay. The QCM Quantitative Cell Migration Assay kit (Chemicon) was used to determine the motility of tumor and endothelial cells. Cells (1 × 10⁴) were resuspended in DMEM with different concentrations of PTK787 and placed into the upper chamber of a 24-well culture plate. After incubating for 24 hours, the upper well was removed and the migrated cells were stained and counted under a microscope. The motility of cells was expressed as a percentage of the number of migrated cells over the total number of inoculated cells.

Western Blot. After treatment, total protein was extracted from the cell lines using radioimmunoprecipitation assay buffer. The protein levels of caspase-9, Bcl-2, cyclin D1, and phosphorylated Akt (P-Akt; mouse anti-rat caspase-9, Bcl-2, cyclin D1, and P-Akt monoclonal antibodies, Cell Signaling Technology, Inc., Beverly, MA), Ral1 and Rho (mouse anti-rat Ral1 and Rho monoclonal antibodies, BD Biosciences PharMingen), VEGF (mouse anti-rat VEGF monoclonal antibody, R&D Systems, Minneapolis, MN), and Flt-1 and Flk-1 (rabbit anti-rat Flt-1 and Flk-1 polyclonal antibodies, Santa Cruz Biotechnology, Santa Cruz, CA) were detected by standard Western blot protocol using 12% SDS-PAGE gel.

Statistical Analysis

Animal survival was analyzed by the log-rank test using the GraphPad Prism software (GraphPad Software, Inc., CA).
San Diego, CA). Comparisons of the tumor size, proliferation index, and number of apoptotic and migrated cells between different groups were done using one-way ANOVA. \( P < 0.05 \) was considered statistically significant.

**Results**

**PTK787 Combined with HAL Prolonged the Survival of Animals**

When no treatment was given, the animals presented with a continuous drop of body weight 2 weeks after sham operation and died within 35 days. Autopsy showed that all the dead rats bore a diffuse-type tumor. HAL alone did not prolong the animal survival, whereas a significant prolongation of animal survival was observed in the group receiving PTK787 treatment alone, and an even longer survival time (median survival of 56 days) was observed in the group with the combined treatment (Fig. 1A).

Before treatment, there was no difference in tumor size among different groups. Fourteen days after treatment, no difference in tumor size was identified between the sham operation and HAL groups. Reduction of tumor size was observed in the group with PTK787 single treatment, and the combined treatment further reduced the size of the tumor (Fig. 1B).

Two days after treatment, scattered areas of necrosis in the tumor tissue were found in the sham operation group, whereas larger areas of necrosis were detected with PTK787 treatment only. Extensive areas of necrosis were observed in the groups with HAL and the combined treatment (Fig. 2A). In the nonnecrotic areas, an increased number of apoptotic cells were detected in the groups with PTK787 single treatment, HAL and the combined treatment, more prominently in the PTK787 only and combined treatment groups (Fig. 2B). Fourteen days after treatment, HAL stimulated an increased expression of von Willebrand factor in the tumor tissues compared with that in the sham control. In the groups with PTK787 single treatment or combined treatment, a dramatic decrease of von Willebrand factor expression was detected (Fig. 2C).

**PTK787 and the Combined Treatment Suppressed the Expression of VEGF and VEGFRs and Inhibited the Viability of both Tumor and Endothelial Cells**

Both tumor and endothelial cells expressed VEGF and VEGFRs, Flt-1 and Flk-1. Hypoxia augmented the expression of VEGF and Flt-1 in both cell lines, whereas a decreased expression of Flk-1 was detected in both cell lines under hypoxic condition, prominently in the tumor cell line. PTK787 either used alone or combined with hypoxia could down-regulate the expression of VEGF and VEGFRs in both cell lines (Fig. 3). In contrast, there was no change in the expression of hypoxia-inducible factor-1a with PTK787 treatment in both cell lines (data not shown).

By using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, decreased viabilities of cells were detected with PTK787 treatment under normoxia in both cell lines. \( O_2 \) (0.1%) also decreased the viability of both types of cells, prominently in the HCC cell line. When PTK787 was administered under the hypoxic condition, further reduction of cell viabilities was detected in both cell lines (Figs. 4A and 5A). In the HCC cell line, an increased number of cells in the \( G_1 \) phase and a decreased number of cells in the \( S \) phase were detected with PTK787 treatment alone or hypoxic treatment alone. However, the combined strategy of PTK787 with hypoxia did not further alter the number of cells in the \( G_1 \) or \( S \) phase. In the endothelial cell line, PTK787 administration induced \( G_1 \)-phase arrest and S-phase suppression only with a high dose (40 \( \mu \)mol/L). Hypoxia alone and the
combined treatment had no obvious effect on the number of endothelial cells in the G₁ or S phase (Fig. 4B and C and Fig. 5B and C). PTK787 treatment either under normoxia or hypoxia could down-regulate the expression of cyclin D1 in the HCC cell line. Hypoxia enhanced the expression of cyclin D1 in the endothelial cell line, but the augmentation could be blocked by adding PTK787 under the hypoxic condition (Figs. 4D and 5D).

**PTK787 Further Increased the Number of Hypoxia-Induced Apoptotic Cells**

The death of cells was determined by Annexin V labeling. PTK787 treatment did not alter the number of apoptotic cells in both cell lines under normoxic condition. However, an increased number of apoptotic cells were observed with hypoxic treatment in both cell lines, and the increase was further enhanced with the administration of PTK787, prominently in the endothelial cells (Fig. 6).

The expression of proapoptotic molecule (caspase-9) and antiapoptotic molecule (Bcl-2) was measured by Western blot. Under normoxia, up-regulation of the cleaved form caspase-9 and down-regulation of Bcl-2 were detected with PTK787 treatment, prominently in the HCC cell line. Hypoxic treatment alone increased the level of cleaved form caspase-9 and reduced the level of Bcl-2 only in the HCC cell line. A further augmentation of cleaved form caspase-9 and a decrease of Bcl-2 expression were detected when hypoxia was combined with PTK787. However, hypoxia alone or the combined approach did not alter the level of cleaved form caspase-9 but down-regulated the expression of Bcl-2 in the endothelial cells (Fig. 6).

**PTK787 Inhibited the Tube Formation Ability of Endothelial Cells under Hypoxic Condition**

Under normoxic condition, endothelial cells were able to form capillary-like structure, whereas this structure was distorted with the administration of PTK787 at the dose of 40 μmol/L. Hypoxia alone did not affect the ability of endothelial cells to form the capillary-like structure, whereas PTK787 administration at the doses of 20 and 40 μmol/L could inhibit the ability of endothelial cells to form capillary-like structure (Fig. 7).

**PTK787 Inhibited the Motility of Tumor and Endothelial Cells under Hypoxic Condition**

Under normoxia, PTK787 administration inhibited the motility of both tumor and endothelial cells across the membrane in a dose-dependent manner. Hypoxia decreased the number of migrated cells in the HCC cell line but increased the number of migrated endothelial cells. In the HCC cell line, an enhanced inhibition of cell migration was found when PTK787 was administrated under hypoxia (Fig. 8), whereas, in the endothelial cell line, PTK787 could suppress the migration of the cells through the membrane under hypoxic condition (Fig. 8).

Under normoxia, PTK787 treatment decreased the expression of Rac1, Rho, and P-Akt in both HCC and endothelial cell lines. Hypoxia decreased the levels of Rac1, Rho, and P-Akt in the tumor cell line, whereas, in the endothelial cell line, it decreased the expression of Rho,
stimulated the expression of Rac1, and had no obvious effect on the expression of P-Akt. When PTK787 was combined with hypoxia, a further reduction of Rac1, Rho, and P-Akt was detected in the HCC cell line, whereas, in the endothelial cell line, the combined treatment downregulated the levels of Rho and P-Akt and reversed the hypoxia-induced up-regulation of Rac1 (Fig. 8).

**Discussion**

The present study revealed that HAL alone did not prolong the survival of animals with HCC. Blockade of VEGFR using PTK787 exhibited limited prolongation of animal survival, whereas, when HAL was combined with PTK787, significant prolongation of animal survival was achieved, suggesting that a combined strategy of induction of tumor cell death by ischemic hypoxia and inhibition of angiogenesis to prevent tumor regrowth could achieve a better therapeutic outcome. This was supported by the histologic findings that massive necrosis in the tumor tissue was induced by HAL, and PTK787 administration caused an increased number of apoptotic cells and decreased the expression of von Willebrand factor and the combined effect led to significant reduction of tumor size several days after treatment.

The dual roles of hypoxia on tumor cell death and proliferation have been reported in some studies, depending largely on the shift of the death-survival balance (32). Similar to the findings in other types of tumors, we also found hypoxia-induced G1-phase arrest and S-phase suppression in the HCC cells (33). However, the endothelial cells did not present with the same phenomenon, suggesting that tumor cells were more sensitive to the alteration of oxygen concentrations than the normal cells. PTK787 administration induced G1-phase arrest and S-phase suppression only under normoxic condition in both cell lines. However, by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, we observed a significant decrease of cell viability with PTK787 treatment under both normoxia and hypoxia, which could not be explained only by the cell cycle arrest. Therefore, we investigated the number of apoptotic cells after treatment and found that enhancement of hypoxia-induced apoptosis could be achieved when PTK787 was combined with hypoxia in both cell lines, more prominently in the tumor cells, indicating that the loss of cell viability in the endothelial cells was mainly due to cell death, whereas, in the HCC cells, both cell cycle arrest and cell death contributed to the decrease of cell viability. The molecular changes supported the above findings. PTK787 treatment suppressed the expression of cyclin D1 in both cell lines under normoxia and further inhibited its expression in the tumor cells under hypoxia. However, hypoxic stress stimulated the expression of cyclin D1 in the endothelial cells, but administration of PTK787 could reverse the hypoxia-induced augmentation of this molecule. Parallel to the findings in the apoptosis assay, up-regulation of proapoptotic molecule caspase-9 and down-regulation of antiapoptotic molecule Bcl-2 were identified by the combined treatment approach in both cell lines.

Although the effects of hypoxia on the angiogenic activities of endothelial cells have been reported in some studies, its molecular basis has not been understood clearly. In the present study, we found that hypoxia did not affect the tube formation ability of endothelial cells. However, administration of PTK787 inhibited the formation of the capillary-like structure under normoxia at the highest dose.

**Figure 3.** The expression of VEGF and VEGFRs with different treatments. Both tumor and endothelial cells expressed VEGF and VEGFRs, Flt-1 and Flk-1. Hypoxia up-regulated the expression of VEGF and Flt-1 in both cell lines but decreased the expression of Flk-1, prominently in the tumor cell line. PTK787 administration either used alone or combined with hypoxia could down-regulate the expression of VEGF and VEGFRs in both cell lines. Representative of three independent experiments.
tested (40 μmol/L), whereas under hypoxia, this inhibitory effect was more prominent at the doses of 20 and 40 μmol/L, suggesting that the integrity of the tubular structure was not dependent on the oxygen alterations, but the hypoxic stress sensitized the endothelial cells to PTK787 treatment.

Unlike some studies showing that hypoxia enhanced the motility of endothelial cells, conflicting results have been reported about its effects on tumor cell migration (34–36). In the present study, we showed a reduced motility of tumor cells under hypoxia. This was supported by the evidence that hypoxia down-regulated...
the expression of migration-related molecules Rac1 and Rho in the tumor cells. On the contrary, hypoxia increased the expression of Rac1 in the endothelial cells, suggesting that hypoxia-enhanced endothelial cell migration might be related to the up-regulation of Rac1. PTK787 administration decreased the expression of Rac1 and Rho in the HCC cell line under both normoxia and hypoxia. Similarly, in the endothelial cell line, PTK787 treatment could also down-regulate the expression of Rho under both normoxia and hypoxia and reverse the hypoxia-induced up-regulation of Rac1, supporting the finding that PTK787 inhibited tumor cell migration under both normoxia and hypoxia and blocked the hypoxia-enhanced migration of endothelial cells. To explore the possible mechanism of PTK787-mediated down-regulation of Rac1 and Rho, we detected the expression of Akt, a molecule that has been identified to interact with the Rac and Rho family (37–39), and found a concomitant expression pattern of P-Akt and Rac1/Rho in both cell lines, indicating that the inhibitory effects of PTK787 might be related to the Akt-related pathways.

Our study showed that, although hypoxia promoted the angiogenic activities of endothelial cells in some aspects, it could also induce cell apoptosis and sensitize endothelial cells to the inhibitory effect of PTK787 on the formation of capillary-like structure and thus achieving an antiangiogenic effect. Hence, PTK787 and hypoxia exerted a synergistic effect on the anticancer activity of each other. Taking the findings in the tumor cells together, dual effects of the combined treatment on cancer cells and angiogenesis could be achieved. This is important for the treatment of HCC patients with an advanced stage using transcatheter arterial chemoembolization, as both induction of tumor cell death and prevention of revascularization after treatment play a crucial role in determining the outcome of patients. The present study did not find any obvious side effects of the combined treatment, in terms of liver function tests (data not shown) and general condition of the animals.

PTK787 was firstly identified as a VEGFR antagonist for the treatment of cancer. However, in the present study, we found that PTK787 could down-regulate the expression of VEGF and VEGFRs at the tested doses in both tumor and
endothelial cell lines but did not alter the expression of hypoxia-inducible factor-1α, an upstream molecule of VEGF, suggesting that, in addition to its effects as a VEGFR antagonist, PTK787 could also achieve its antitumor cell and antiangiogenic effects through direct suppression of VEGF and VEGFR expression and subsequent blockade of the VEGF-VEGFR signaling pathway. However, PTK787 might function on other receptor families expressed on endothelial cells (40). Therefore, we believed that the inhibitory effects of PTK787 on tumor and endothelial cells were through both VEGF-VEGFR-dependent and VEGF-VEGFR-independent pathways. However, further studies are still needed to explore the molecular basis of PTK787-mediated suppression of VEGF and VEGFRs.

Based on our previous finding about the effects of PTK787 on human HCC cell lines (29) and study the study of Lee et al. (41) showing that oral feeding of PTK787 at the dose of 50 mg/kg yielded 62.4 (±16.0) h μmol/L plasma exposure, we applied PTK787 at the concentration of μmol/L, to the tumor and endothelial cell lines, and found inhibitory effects on cell cycle progression and induction of cell apoptosis in both cell lines. We have also tested the effects of PTK787 on a human umbilical vascular endothelial cell and a mouse tumor-derived endothelial cell

Figure 7. The effect of PTK787 and hypoxia on the tube formation ability of endothelial cells. A, PTK787 inhibited the formation of the capillary-like structure at the highest dose tested (40 μmol/L) under normoxia (B), whereas a lower dose of PTK787 (20 μmol/L) could achieve a similar effect under hypoxia. Representative of three independent experiments. NT, sham operation (no treatment).

Figure 8. The effects of hypoxia and PTK787 combined with hypoxia on tumor and endothelial cell migration. A, in the tumor cell line, hypoxia inhibited the motility of tumor cells through the membrane, and the suppression was further enhanced by the administration of PTK787. The expression of Rac1, Rho, and P-Akt was reduced with hypoxia alone and the combined approach. B, in the endothelial cell line, hypoxia enhanced the motility of endothelial cells across the membrane, whereas administration of PTK787 could reverse the hypoxia-enhanced migration. Hypoxia up-regulated the expression of Rac1, but it was reduced by the administration of PTK787. The expression of Rho was decreased with the hypoxic stimulation, and the down-regulation was further enhanced by the administration of PTK787. Hypoxia had no obvious effect on the expression of P-Akt, but the combined treatment significantly down-regulated the expression of P-Akt. Representative of four independent experiments. *, P < 0.05, compared with NT under normoxia; #, <0.05, compared with NT under hypoxia. NT, sham operation (no treatment).
line (MILE SVEN 1) and found that PTK787 could achieve similar antiangiogenic effects on the mouse tumor-derived endothelial cell line with the same doses of the present study, but with different doses in the human umbilical vascular endothelial cell line (data not shown), implying that the sensitivities to PTK787 varied among different cell lines. In the clinical setting, although promising data has been obtained using this drug for the treatment of cancer, evaluation for therapeutic efficacy remains a problem (42), as it is difficult to determine the optimal doses for drug action and maximal reduction of side effects. Therefore, more clinical trials are ongoing to assess the ultimate doses for the treatment of different cancers. The present in vivo animal data revealed that the dose of 50 mg/kg PTK787 oral administration daily could enhance the efficacy of hypoxia without any observable side effects. Therefore, based on these findings, a clinical trial by the combination of PTK787 with transcatheter arterial chemoembolization is justified.

In conclusion, VEGF receptor blockade using PTK787 combined with hypoxia could achieve a better therapeutic efficacy than hypoxia alone through both antitumor cell and antiangiogenic effects. The antitumor cell effect was more dependent on suppression of cell proliferation, induction of cell death, and inhibition of tumor cell migration, whereas the antiangiogenic effect was more dependent on induction of cell death, inhibition of tube formation ability, and suppression of hypoxia-enhanced motility of endothelial cells.

References


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