Introduction

Lung cancer causes more deaths worldwide each year than any other type of cancer. One of the main reasons for poor prognosis of lung cancer patients is that this disease frequently metastasizes to multiple organs. Unfortunately, more than 70% of lung cancer patient have advanced stage disease at the time of diagnosis [1]. Although intensive efforts have been made to treat lung cancer, it is still difficult to control lung cancer metastasis. Therefore, it is very important to investigate and understand the molecular and biological mechanisms that contribute lung cancer metastasis, which is the most devastating complication of lung cancer, and which is responsible for 90% of all associated deaths [2].

Recently, several studies have indicated the importance of gender difference in human lung cancers. For example, there are gender differences in the incidence and mortality of lung cancer, and particularly the delayed increase and then leveling off of the lung cancer risk in women in comparison to men [3–5]. The therapeutic response to chemotherapy is also different in association with gender [6]. These findings suggest that gender difference is one of the key factors that define the lung cancer progression.

Sex steroids and their receptors, such as androgen receptor (AR) and estrogen receptor (ER), seem to be the most important factors that affect gender difference. Several lines of evidence indicate the involvement of sex steroids and their receptors in the development of several cancers, including lung cancer. Niikawa et al. reported that the tissue concentration of estradiol in human non-small cell lung cancer (NSCLC) is significantly higher than normal lung tissues and that estradiol significantly increased the cell proliferation of human NSCLC, A549 cells stably expressing ER [7]. Nishio et al. demonstrated an epidermal growth factor receptor-tyrosine kinase inhibitor, gefitinib, to significantly suppress the serum androgen levels and that gefitinib responders had significantly lower androgen levels than non-responders in female NSCLC patients [8]. Moreover, using an orthotopic lung carcinoma model, Montgrain et al. reported that tumor burden was significantly less in female mice possibly because of negative regulation of parathyroid hormone-related protein (PTHrP) by androgen in males [9]. These results suggest that sex steroids and their receptors play an important role in the gender difference in NSCLC. However, despite the accumulated evidence in NSCLC, the involvement of sex hormone in the development and progression of human small cell lung cancer (SCLC) still remains uncertain. Furthermore, although metastasis is one of the key biological phenomenon in lung cancer progression, the relationship between gender difference and lung cancer metastasis has not been thoroughly investigated.

The present study used a multiple organ metastasis model in natural killer (NK) cell-depleted severe immunodeficient (SCID) mice to evaluate the gender difference in multiple organ metastases produced by human SCLC, SBC-5 cells.

Materials and methods

Cell culture

The human SCLC cell lines, SBC-3 and SBC-5, were kindly provided by Drs. M. Tanimoto and K. Kiura (Okayama University, Okayama, Japan). The human prostate cancer cell line, PC-3, was kindly provided by Dr. T. Fukumori (The University of Tokushima, Tokushima, Japan). The human breast cancer cell line, MCF-7, was purchased from American Type Culture Collection (Manassas, VA). SBC-5 was maintained in RPMI 1640 (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY), penicillin (100 U/ml), and streptomycin (50 μg/ml). SBC-3, PC-3 and MCF-7 were maintained in MEM (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% fetal bovine serum, penicillin, and streptomycin. All cell lines were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air.

Reagents

Bicalutamide, an orally active androgen receptor antagonist, was purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). Testosterone and 5α-androstan-17β-OL-3-one (dehydro-testosterone: DHT), a natural metabolite of testosterone (3 times more potent than testosterone), and β-estradiol (E2), which represents the major estrogen in humans, were purchased from SIGMA Aldrich (St. Louis, MO). Anti-mouse IL-2 receptor β chain monoclonal antibody, TM-β1 (IgG2b), was kindly supplied by Drs. M. Miyasaka and T. Tanaka (Osaka University, Osaka, Japan).

Reverse transcription-polymerase chain reaction

The total RNA was extracted from the cultured cells using the RNeasy mini kit (Qiagen, Valencia, CA). Aliquots of 2000 ng RNA were reverse transcribed to cDNA by using Kit for omniscript RT kit. The cDNA products were subjected to reverse transcription polymerase chain reaction (RT-PCR) amplification. The primer sequences were: human AR: sense 5′-TGG ACA CGA CAA CAA CCA GCC-3′ and antisense 5′-CTG GTA GAA GCG TCT TGA GC-3′, human ERα: sense 5′-CAA GGC ACT GAC CAT CTG GT-3′ and antisense 5′-CAA GGC ACT GAC CAT CTG GT-3′, human ERβ: sense 5′-CTG TTA CTG GTC

CAG GTT CA-3′ and antisense 5′-CCA GCT GAT CAT GTG TAC CA-3′, human PTHrP: sense 5′-AGA CGA TGC AGC GGA GAC-3′ and antisense 5′-GCC GTA AAT CTT GGA TGG AC-3′, human β-actin: sense 5′-AAG AGA GGC ATC CTC ACC CT-3′ and antisense 5′-TAC ATG GCT GGG GTG TTG AA-3′. The complementary DNAs were amplified using 0.1 μl of Hot Star Taq DNA Polymerase (Qiagen, Valencia, CA) for 33 polymerase chain reaction (PCR) cycles in ER, AR and for 25 PCR cycles in PTHrP (initial Taq activation at 94°C for 8 min, denaturing at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 30 s, followed by an additional extension cycle at 72°C for 7 min). The amplified products were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining with ultraviolet light illumination.

Animals

Male and Female S EB-17/Icr-SCID mice, aged 5–6 weeks were obtained from CLEA Japan (Osaka, Japan) and maintained under specific pathogen-free conditions throughout the experiment. All experiments were performed according to the guidelines of The University of Tokushima.

Multiple organ metastasis model in NK cell-depleted SCID mice using SBC-5 cells and the effects of castration and bicalutamide treatment

A multiple organ metastasis model using NK cell-depleted SCID mice was previously established to investigate the metastatic feature of SBC-5 cells in vivo, (10). Briefly, to facilitate the metastasis of SBC-5 cells, NK cells were depleted in SCID mice. For this, TM-β1 monoclonal antibody (300 μg/300 μl PBS/mouse) was injected i.p. into SCID mice 2 days before tumor cell inoculation. SBC-5 cells in the subconfluent condition were harvested and washed with Ca^{2+}- and Mg^{2+}-free PBS (CMF-PBS). SBC-5 cells (1×10^6 cells/300 μl) were injected into the lateral tail vein of mice on day 0.

Four to five weeks after the tumor cell inoculation, the mice were anesthetized with an i.p. injection of pentobarbital (0.5 mg/body), and X-ray photographs of the mice were taken to evaluate the presence of bone metastasis. Next, blood specimens were collected by cardiac puncture, and all major organs were removed. The lungs were fixed in Bouin's solution for 24 h. The number of metastatic lesions on the surface of the major organs was counted macroscopically. The blood was centrifuged 5000 rpm for 15 min, and sera was collected and kept frozen until future use. For the experiment with either castration or bicalutamide treatment, the number of metastasis were compared between control male and castrated male, or between control male and bicalutamide treated male. The mice in the castrated group were castrated at 5–8 days before TM-β1 treatment. In bicalutamide treatment group, the drug was given orally at 0.5 mg/mouse daily as a suspension in 0.5% Tween 80 from day 3 to day 35.

Immunohistochemical analyses

The hind limbs of the mice were taken and fixed in 10% formalin. The bone specimens were decalcified in 10% EDTA solution for 1 week and then embedded in paraffin. The paraffin-embedded tissue samples were cut in 3-μm sections and picked up on slides. Tartrate-resistant acid phosphatase (TRAP) staining was performed using a Sigma Diagnostics Acid Phosphatase Kit (Sigma Diagnostics, St. Louis, MO) for the detection of osteoclasts. The number of TRAP-positive osteoclasts at the tumor-bone interface in each slide was counted under a microscope in five random fields at 200× magnification.

In vitro proliferation assay

Cell proliferation was measured by the MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium] dye reduction method [10]. Briefly, SBC-5 cells (5×10^3 cells/100 μl/well) were seeded into each well of 96-well plates and treated with various concentrations of DHT, E2 or bicalutamide. After 72 h incubation at 37°C, 50 μl of MTT stock solution (2.5 mg/ml) was added to each well, and the cells were further incubated for 2 h at 37°C. Then, 100 μl of DMSO was added to dissolve the dark blue crystals. Absorbance was measured with an MTP-32 Microplate Reader (Corona Electric, Ibaragi, Japan) at test and reference wavelengths of 550 and 630 nm, respectively.

Analysis of PTHrP expression in vivo and in vitro

The levels of PTHrP in the mouse sera were determined using a radioimmunoassay (SRL Co., Tokushima, Japan). SBC-5 cells were plated into 6-well tissue culture plates (5×10^5 cells/2 ml/well) for the in vitro studies. After 24 h incubation, various concentrations of DHT or E2 were added at final concentration of 0, 0.1, 1 and 10 nM. After 12, 24, and 48 h, culture supernatants were harvested and the levels of PTHrP were determined by a radioimmunoassay (SRL Co., Tokushima, Japan). PTHrP mRNA expression was examined by RT-PCR.

Statistical analyses

The Mann–Whitney U test was used to determine the significance of differences in the number of metastases into

multiple organs (the bone, liver, and lung) and TRAP-positive cells between each group. One-Way ANOVA was used to determine the significance of differences in the levels of PTHrP in culture supernatant. The significance of differences in the levels of PTHrP in mouse serum was analyzed by Student's *t*-test (two-tailed). $P < 0.05$ was considered to be significant in all experiments.

Results

Analysis of AR and ER expression in human SCLC cells

The gene expression status of AR and ER was analyzed in human SCLC cells. RT-PCR analyses revealed that human SCLC cell line, SBC-5 expressed mRNA for AR and ER-β, but not ER-α. On the other hand, another human SCLC cell line, SBC-3 expressed only ERs and did not express AR (Fig. 1). The SBC-5 cell line was selected for further studies because it expressed both AR and ER. MCF-7 and PC-3 cells were used as positive controls.

Evaluation of gender difference in multiple organ metastases produced by SBC-5 cells in NK cell-depleted SCID mice

A previous study established an osteolytic bone metastasis model with multiple organ dissemination in NK cell-depleted SCID mice using SBC-5 cells, which highly expressed PTHrP [10]. This model was used to determine whether the gender difference affects the multiple organ metastasis profile produced by SBC-5 cells. Intravenously inoculated SBC-5 cells produced multiple organ metastases (lungs, liver and bone). Interestingly, only the number of bone metastasis was significantly increased in female mice in comparison to males, whereas no difference was observed in the metastases to the lungs and liver (Table 1).

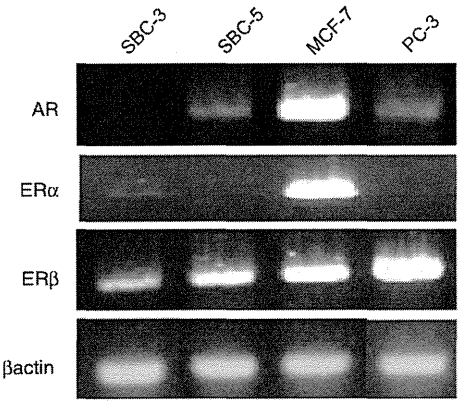

Fig. 1 The analysis of hormone receptor expression in human SCLC cells. The expression levels of AR, ERα and ERβ in SBC-3 and SBC-5 cells were determined by RT-PCR as described in "Materials and methods". Data are representative of two independent experiments with similar results. MCF-7 cells and PC-3 cells were used as positive control for ER and AR, respectively

Effect of castration on multiple organ metastases by SBC-5 cells in NK cell-depleted SCID mice

The effect of castration on bone metastasis by SBC-5 cells in male mice was examined to confirm the gender difference in bone metastasis. The number of bone metastases significantly increased in the castrated group in comparison to the control group (Table 2), thus suggesting that the down regulation of androgen by castration increased the incidence of bone metastasis by SBC-5 cells. Liver and lung metastases were increased in the castrated group in some experiments, but it was not reproducible.

Effect of androgen blockade on multiple organ metastases by SBC-5 cells in NK cell-depleted SCID mice

SBC-5 cell-bearing male mice were treated with an androgen receptor antagonist, bicalutamide. As shown in Table 3, bone metastasis was significantly increased in the bicalutamide treated group in comparison to the control group, while no difference was seen on the metastases to the visceral organs (Table 3). These in vivo results suggest that the balance of sex steroid plays an important role in osteolytic lung cancer bone metastasis.

Assessment of the number of osteoclasts in bone lesions in SBC-5 cell-bearing mice

The number of osteoclasts in the bone lesions was examined since osteoclasts are known to play the main role in osteolytic bone metastasis. An immunohistochemical analysis revealed that the number of osteoclasts, which were detected as TRAP positive cells, was significantly increased in the lesions of osteolytic bone metastases in female mice, castrated male mice, or bicalutamide treated male mice in comparison to the control male mice (Fig. 2).

Changes in serum PTHrP levels in the bone metastasis of SBC-5 cell-bearing male and female mice

SBC-5 cells produce PTHrP, and the PTHrP plays an important role in bone metastasis induced by SBC-5 cells [10]. The PTHrP concentration in the sera of SBC-5 cell-bearing mice was evaluated to determine if PTHrP is involved in the gender difference of bone metastasis. The serum concentration of PTHrP tended to be higher in female mice, castrated mice, or bicalutamide-treated mice

Table 1 Gender difference of metastatic features of human SCLC, SBC-5 cells

Gender	Bone			Liver					Lung				
	Number of metastasis			Weight (mg)		Number of metastasis			Weight (mg)		Number of metastasis		
	Inc.	Med.	Range	Med.	Range	Inc.	Med.	Range	Med.	Range	Inc.	Med.	Range
Experiment 1													
Male	5/5	3	2–4	1372	970–1480	5/5	42	14–54	174	145–180	5/5	17	15–35
Female	5/5	5	4–6*	1141	913–1435	5/5	29	4–56	167	128–185	5/5	8	6–48
Experiment 2													
Male	5/5	5	4–6	1621	1111–2150	5/5	71	26–82	185	170–217	5/5	13	5–23
Female	5/5	8	6–13*	1118	948–1461	5/5	48	24–62	169	131–190	5/5	13	7–25

Inc. Incidence, *Med.* Median

SBC-5 cells (1×10^6 cells/mouse) were inoculated i.v. into NK cell-depleted male or female SCID mice on day 0 (five mice per group). The metastatic profile was evaluated 35 days after cell inoculation

Mann–Whitney *U* test was used to determine the significance of differences

* Statistically significant difference compared with male group ($P < 0.05$)

Table 2 Effect of castration on multiple organ metastases by human SCLC, SBC-5 cells

Group	Bone			Liver					Lung				
	Number of metastasis			Weight (mg)		Number of metastasis			Weight (mg)		Number of metastasis		
	Inc.	Med.	Range	Med.	Range	Inc.	Med.	Range	Med.	Range	Inc.	Med.	Range
Experiment 1													
Control	4/5	2	0–5	1640	1200–2230	4/4	83	5–150	195	140–210	3/4	15	0–27
Castration	6/6	7	5–8*	3550	860–8060*	5/5	150	5–150	250	180–310	4/5	36	0–70
Experiment 2													
Control	7/7	3	2–4	1450	1220–2890	7/7	46	28–150	220	190–270	7/7	25	5–32
Castration	8/8	6	5–7*	1085	900–5350	8/8	23.5	12–150	230	210–270	8/8	52.5	7–98*

Inc. Incidence, *Med.* Median

Male mice were castrated in castration group on day 5. SBC-5 cells (1×10^6 cells/mouse) were inoculated i.v. on day 0 in both group. The metastatic profile was evaluated 35 days after cell inoculation

Mann–Whitney *U* test was used to determine the significance of differences

* Statistically significant difference compared with control group ($P < 0.05$)

Table 3 Effect of bicalutamide on multiple organ metastases by human SCLC, SBC-5 cells

Group	Bone			Liver					Lung				
	Number of metastasis			Weight (mg)		Number of metastasis			Weight (mg)		Number of metastasis		
	Inc.	Med.	Range	Med.	Range	Inc.	Med.	Range	Med.	Range	Inc.	Med.	Range
Experiment 1													
Control	4/4	3.5	3–4	1715	1620–2710	4/4	54	36–80	245	210–270	4/4	18.5	13–25
Bicalu.	5/5	7	4–7 *	1730	1540–2670	5/5	53	37–57	240	210–720	5/5	19	7–26
Experiment 2													
Control	3/3	4	4–4	1820	1550–2120	3/3	150	4–150	200	190–210	3/3	40	5–61
Bicalu.	8/8	6.5	5–8 *	2210	1420–2660	8/8	150	70–150	210	190–240	8/8	26.5	9–57

Inc. Incidence, *Med.* Median, *bicalu.* bicalutamide

SBC-5 cells (1×10^6 cells/mouse) were inoculated i.v. into NK cell-deleted male SCID mice on day 0 in each group. Bicalutamide (25 mg/kg) or vehicle was given p.o. every day starts on day 3. The metastatic profile was evaluated 35 days after cell inoculation

Mann–Whitney *U* test was used to determine the significance of differences

* Statistically significant difference compared with vehicle group ($P < 0.05$)

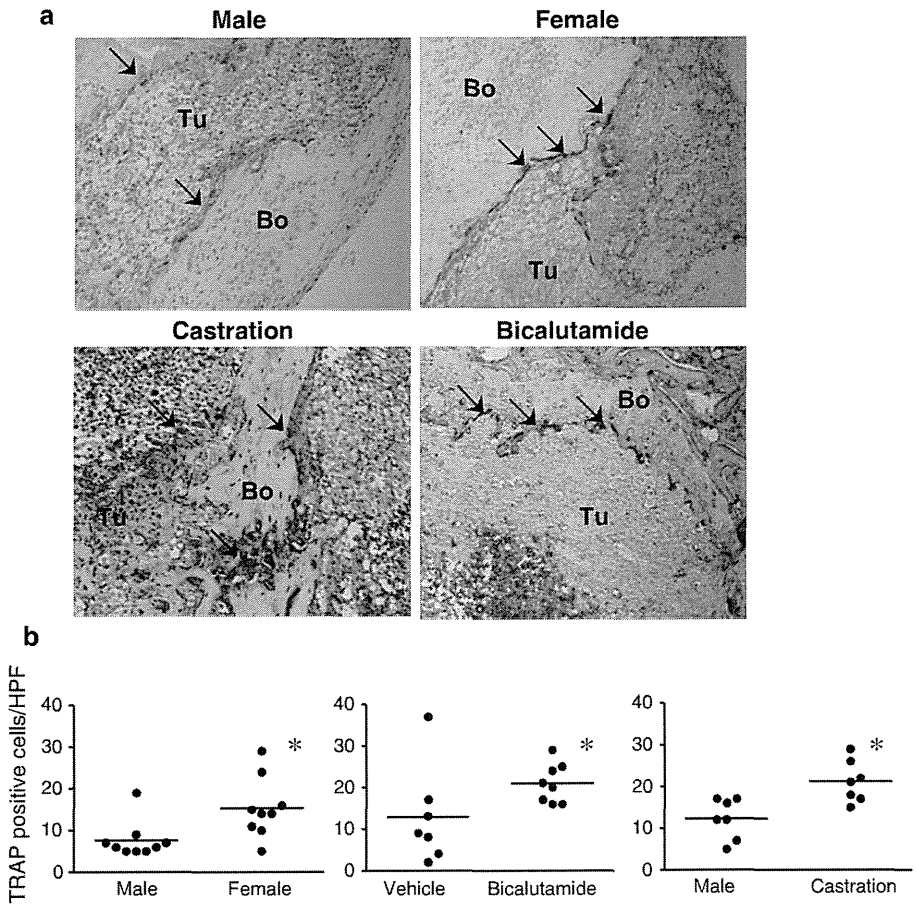

Fig. 2 An immunohistochemical analysis of metastatic bone lesions produced by SBC-5 cells. **a** Osteoclasts in the bone lesion were determined by TRAP staining. The representative picture from each group is shown. "Tu" indicates tumors, and "Bo" indicates bone. The arrows indicate osteoclasts. (**b**) The number of osteoclasts was determined in each experiment. * $P < 0.05$

than in control male mice, but the differences were not significant (Fig. 3).

Evaluation of the effect of sex steroids on the proliferation and PTHrP expression in human SCLC, SBC-5 cells in vitro

The direct effect of sex steroids on the proliferation of SBC-5 cells was investigated in vitro. DHT and E2 had no effect on the proliferation of SBC-5 cells in the concentration of up to 100 μM (Fig. 4a, b). In addition, bicalutamide also had no in vitro effect on the proliferation of SBC-5 cells (Fig. 4c).

The effect of sex steroids on the PTHrP production in SBC-5 cells was further investigated in vitro. DHT and E2 had no obvious effect on the PTHrP production examined with radioimmunoassay (Fig. 5). Similarly, an RT-PCR analysis showed no significant change of PTHrP mRNA expression irrespective of the addition of sex steroids (Fig. 5). These results indicated that sex steroids had no direct effect on the expression and production of PTHrP in SBC-5 cells.

Discussion

The present study showed the number of lung cancer bone metastasis to bone, but not to visceral organs by human SCLC, SBC-5 cells to be significantly greater in female SCID mice in comparison to male mice. This is the first report demonstrating a gender difference of bone metastasis formation in human small cell lung cancer cells.

Metastasis to multiple organs is a critical problem for lung cancer patients. In particular, the most serious problem in the management of lung cancer patients is metastatic spread to various distant organs, and it is already established (microscopically or macroscopically) by the time of diagnosis in many cases. The skeleton is one of the most common sites of metastasis in patients with lung cancer. The incidence of bone metastasis in lung cancer patients is 30–40% [11–13]. Bone metastases frequently raise serious complications such as skeletal-related events, including fracture, spinal cord compression, and hypercalcemia, which markedly deteriorate the quality of life in cancer patients. Therefore, the prevention and proper management of bone metastasis is essential [14], and better

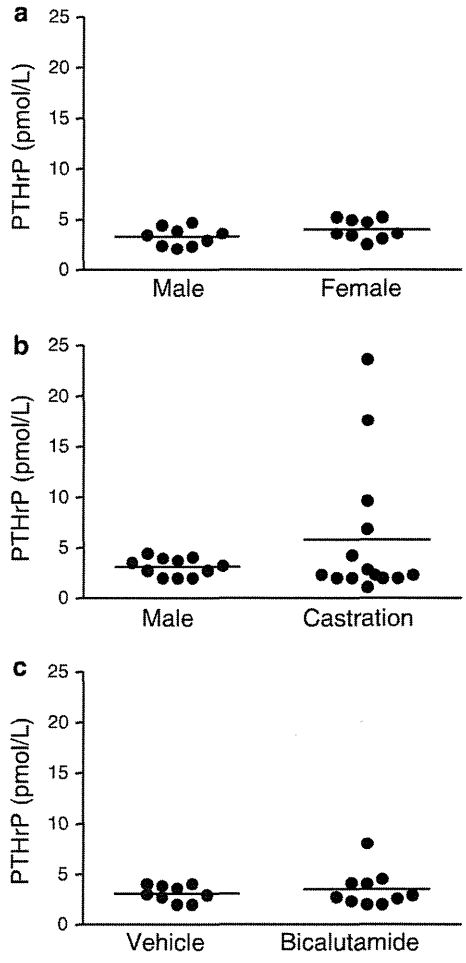

Fig. 3 An analysis of serum concentration of PTHrP in SBC-5-bearing mice. Serum concentration of PTHrP in female mice (**a**), or castrated mice (**b**), or bicalutamide-treated mice (**c**) was determined by radioimmunoassay

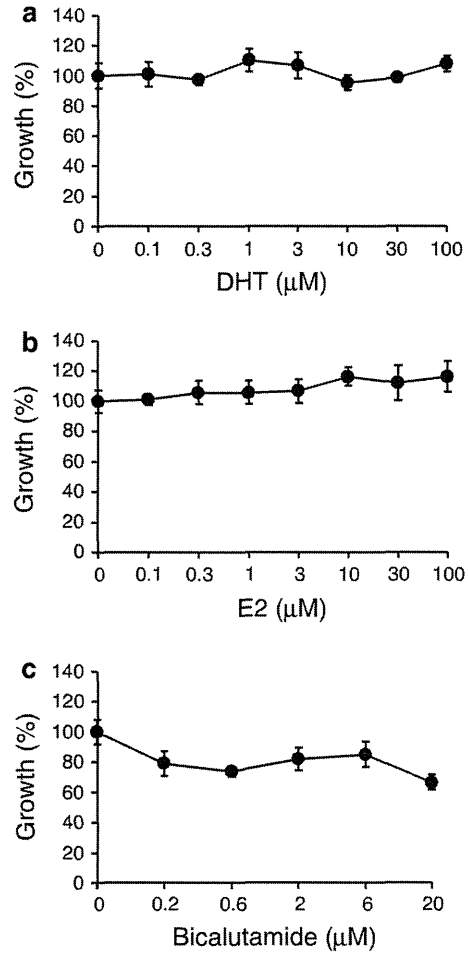

Fig. 4 The effect of sex steroids and bicalutamide on the proliferation of SBC-5 cells. SBC-5 cells (5×10^3 cells/100 μl/well) were incubated with various concentrations of testosterone (DHT), estradiol (E2), or bicalutamide. The effect of DHT (**a**), E2 (**b**), or bicalutamide (**c**) on the proliferation of SBC-5 cells was determined by MTT assay as described in the "Materials and methods"

understanding of biology and mechanism of cancer bone metastasis is warranted.

The gender difference plays a major role in bone metabolism, and androgen and estrogen are critical regulators of bone turnover to maintain bone mass. Estrogen deficiency, which is usually seen in menopausal women, induces increased bone resorption and results in osteoporosis. Testosterone suppressed bone resorption to maintain bone mass in an estrogen-independent manner [15] and castration induces increased bone turnover and reduced bone mineral density in human [16] and rat [17]. Fukusato et al. reported that the bone metastasis of hepatocellular carcinoma might be prevalent in male patients with liver cirrhosis [18], and other reports showed that estrogen or androgen affects the secretion of PTHrP or bone metastasis in various cancer cell lines [19–21]. In addition to the results from in vivo experiments, these findings suggest that the balance of sex hormones plays an important role in cancer bone metastasis as well as normal bone metabolism.

However, the degree of bone metastasis due to the unbalance of sex steroids and its receptors is contradictory. For instance, Rabbani et al. demonstrated that estradiol caused a marked decrease in breast cancer cell growth and PTHrP production, and the overexpression of ER results in the decrease of tumor growth and bone metastasis [20], thus suggesting that estrogen-ER axis contributes to the decrease of breast cancer bone metastasis. On the contrary, the results from the current in vivo experiments suggest that the relative increase of estrogen in female mice or male mice with an androgen blockade contribute to the increase of lung cancer bone metastasis. The study performed by Zhau et al. may support the current data, showing that the androgen-repressed state may be central to prostate cancer progression and metastasis [22]. These differences may be due to the difference of cell types and gene expression profile of cancer cells. Martinez et al. have

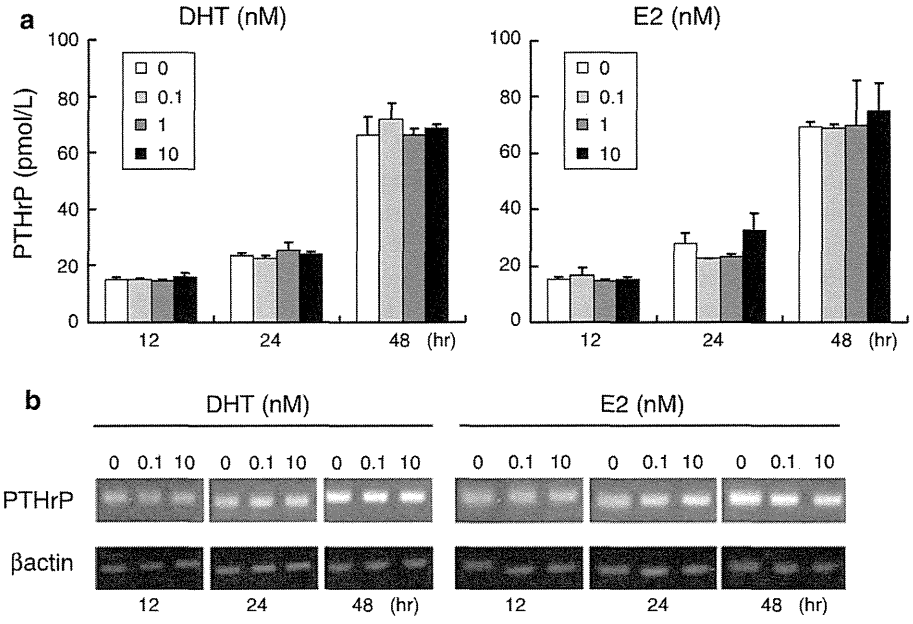

Fig. 5 Effect of sex steroids on the production of PTHrP in SBC-5 cells in vitro. SBC-5 cells (5×10^5/well) were incubated with various concentrations of testosterone (DHT) (**a**) and estradiol (E2) (**b**), and culture supernatants were harvested at the indicated time points. The concentrations of PTHrP in culture supernatants were determined by radioimmunoassay. PTHrP mRNA expression in response to various concentrations of DHT (**a**) or E2 (**b**) was examined by RT-PCR. Data are representative of two independent experiments with similar results

shown that estradiol receptor-positive cells acquire a more aggressive phenotype than estradiol receptor-negative cells when cultured on an extracellular matrix produced by an osseous cell line [23]. This report suggests that the state of not only the sex steroids but also their receptors is important in the formation of cancer bone metastasis. Further study is needed to clarify the importance of sex steroids and their receptors in the field of cancer bone metastasis.

Bone destruction caused by bone metastasis is mediated by various factors produced or induced by tumor cells and/or host cells that stimulate the formation and activation of osteoclasts, the normal bone-resorbing cells. Several factors, including interleukin (IL)-6, receptor activator of nuclear factor-κB (RANK), RANK ligand (RANKL), macrophage inflammatory protein-1α and PTHrP have been implicated as factors that enhance osteoclast activation and bone destruction in malignant diseases [24]. PTHrP has also been intensively reported to indirectly activate osteoclasts by induction or stimulation of RANKL expression by osteoblasts and hence augment bone resorption. In addition, treatment with anti-PTHrP neutralizing antibody inhibits the development of bone metastasis by SBC-5 cells in NK cell-depleted SCID mice, indicating that PTHrP is responsible, at least in part, for the production of bone metastasis by SBC-5 cells [10].

Montgrain et al. has recently shown that PTHrP is responsible for gender difference in NSCLC progression. Experimental lung carcinomas in female mice contain approximately 3 times more PTHrP than in male mice, and androgen treatment reduces PTHrP expression in lung cancer cell lines [25]. This study indicates that PTHrP in tumor is regulated by sex steroids. However, androgen (DHT) and estrogen (E2) had no obvious effect on cell growth and PTHrP production of SBC-5 cells in vitro in the current study (Fig. 4), and no significant gender difference was observed on the PTHrP concentration in the sera of SBC-5 cell-bearing mice (Fig. 3). Nevertheless, the number of osteoclasts in osteolytic bone metastatic lesions was significantly increased in SBC-5 cell-bearing female mice (Fig. 2), suggesting the possibility of sex steroid-dependent regulation of osteoclastogenesis by other factors such as IL-6, osteoprotegerin, or transforming growth factor β. Indeed, Bellido et al. have demonstrated that androgen inhibited the expression of the IL-6 gene, and that IL-6 mediates upregulation of osteoclastogenesis [26]. Further

Table 4 Summary of bone metastasis profile

Gender	Castration	Bicalutamide	Bone metastasis	Osteoclasts	Serum PTHrP
Male	−	−	+	+	+
Female	−	−	++	++	+
Male	+	−	++	++	+
Male	−	+	++	++	+

SBC-5 cells (1 × 106 cells/mouse) were inoculated i.v. into NK cell-deleted male SCID mice on day 0 in each group. Castration was performed on day 5. Bicalutamide (25 mg/kg) was given p.o. every day starts on day 3. The metastatic profile was evaluated 35 days after cell inoculation

studies are warranted to elucidate the effect of sex steroids on the interaction between tumor cells and the bone microenvironment.

In conclusion, this study provided the first evidence of a gender difference of bone metastasis formation in human SCLC (data summarized in Table 4). An androgen blockade induced significant increase of bone metastases, thus indicating that the balance of sex steroids is one of the key factors which affects on the interaction between cancer cell and host cells in the bone microenvironment and it thereby enhances the "vicious cycle" of bone metastases.

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References

1. Sone S, Yano S (2007) Molecular pathogenesis and its therapeutic modalities of lung cancer metastasis to bone. Cancer Metastasis Rev 26:685–689
2. Vicent S, Luis-Ravelo D, Antón I et al (2008) A novel lung cancer signature mediates metastatic bone colonization by a dual mechanism. Cancer Res 68:2275–2285
3. Jemal A, Thun MJ, Ries L et al (2008) Annual report to the nation on the status of cancer, 1975–2005, featuring trends in lung cancer, tobacco use, and tobacco control. J Natl Cancer Inst 100:1672–1694
4. Ou SH, Ziogas A, Zell JA et al (2009) Prognostic factors for survival in extensive stage small cell lung cancer (ED-SCLC): the importance of smoking history, socioeconomic and marital statuses, and ethnicity. J Thorac Oncol 4:37–43
5. Asamura H, Goya T, Koshiishi Y et al (2008) A Japanese lung cancer registry study: prognosis of 13, 010 resected lung cancers. J Thorac Oncol 3:46–52
6. Dongiovanni D, Daniele L, Barone C et al (2008) Gefitinib (ZD1839): therapy in selected patients with non-small cell lung cancer (NSCLC)? Lung Cancer 61:73–81
7. Niikawa H, Suzuki T, Miki Y et al (2008) Intratumoral estrogens and estrogen receptors in human non-small cell lung carcinoma. Clin Cancer Res 14:4417–4426
8. Nishio M, Ohyanagi F, Horiike A et al (2005) Gefitinib treatment affects androgen levels in non-small-cell lung cancer patients. Br J Cancer 92:1877–1880
9. Montgrain PR, Quintana R, Rascon Y et al (2007) Parathyroid hormone-related protein varies with sex and androgen status in nonsmall cell lung cancer. Cancer 110:1313–1320
10. Miki T, Yano S, Hanibuchi M et al (2004) Parathyroid hormone-related protein (PTHrP) is responsible for production of bone metastasis, but not visceral metastasis, by human small cell lung cancer SBC-5 cells in natural killer cell-depleted SCID mice. Int J Cancer 108:511–515
11. Mohla S, Weilbacher KN, Cher ML et al (2003) Third North American symposium on skeletal complications of malignancy: summary of the scientific sessions. Cancer 97:719–725
12. Tsuya A, Fukuoka M (2008) Bone metastasis in lung cancer. Club Calcium 18:455–459
13. Coleman RE (1997) Skeletal complications of malignancy. Cancer 80(Suppl 8):1588–1594
14. Coleman RE, Guise TA, Lipton A et al (2008) Advancing treatment for metastatic bone cancer: consensus recommendations from the Second Cambridge Conference. Clin Cancer Res 14:6387–6395
15. Matsumoto C, Inada M, Toda K et al (2006) Estrogen and androgen play distinct roles in bone turnover in male mice before and after reaching sexual maturity. Bone 38:220–226
16. Yamada Y, Takahashi S, Fujimura T et al (2008) The effect of combined androgen blockade on bone turnover and bone mineral density in men with prostate cancer. Osteoporos Int 19:321–327
17. Allan G, Lai MT, Sbriscia T et al (2007) A selective androgen receptor modulator that reduces prostate tumor size and prevents orchidectomy-induced bone loss in rats. J Steroid Biochem Mol Biol 103:76–83
18. Fukusato T, Aoyama H, Mori W (1989) Age and sex differences in bone metastasis of hepatocellular carcinoma in Japanese autopsy cases. Gastroenterol Jpn 24:127–134
19. Funk JL, Wei H (1998) Regulation of parathyroid hormone-related protein expression in MCF-7 breast carcinoma cells by estrogen and antiestrogens. Biochem Biophys Res Commun 251:849–854
20. Rabbani SA, Khalili P, Arakelian A et al (2005) Regulation of parathyroid hormone-related peptide by estradiol: effect on tumor growth and metastasis in vitro and in vivo. Endocrinology 146:2885–2894
21. Pizzi H, Gladu J, Carpio L et al (2003) Androgen regulation of parathyroid hormone-related peptide production in human prostate cancer cells. Endocrinology 144:858–867
22. Zhau HY, Chang SM, Chen BQ et al (1996) Androgen-repressed phenotype in human prostate cancer. Proc Natl Acad Sci USA 93(26):15152–15157
23. Martinez J, Fuentes M, Cambiazo V et al (1999) Bone extracellular matrix stimulates invasiveness of estrogen-responsive human mammary MCF-7 cells. Int J Cancer 83(2):278–282
24. Hall CL, Keller ET (2006) The role of Wnts in bone metastases. Cancer Metastasis Rev 25:551–558
25. Montgrain PR, Quintana R, Rascon Y et al (2007) Parathyroid hormone-related protein caries with sex and androgen status in nonsmall cell lung cancer. Cancer 110(6):1313–1320
26. Bellido T, Jilka RL, Boyce BF (1995) Regulation of interleukin-6, osteoclastogenesis, and bone mass by androgens. The role of the androgen receptor. J Clin Invest 95(6):2886–2895

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CASE REPORT

Metastatic renal cell carcinoma complicated with diffuse alveolar hemorrhage: a rare adverse effect of sunitinib

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Abstract We report the case of a 67-year-old man with metastatic papillary renal cell carcinoma (RCC) who developed bloody sputum after the administration of sunitinib. Chest computed tomography revealed diffuse ground-glass opacity lesions, and bloody bronchoalveolar lavage fluid was obtained by flexible bronchoscopy. The abnormal shadows promptly regressed after withdrawal of sunitinib. In four cycles of sunitinib treatment, he suffered from controllable diffuse alveolar hemorrhage. Finally, he died of respiratory failure 8 months after onset. This is the first case report of diffuse alveolar hemorrhage as an adverse effect of sunitinib in metastatic papillary RCC. Care should be taken with pulmonary hemorrhage in the use of anti-angiogenesis agents in not only squamous cell lung cancer, but also metastatic lung tumors.

Keywords Sunitinib · Alveolar hemorrhage · Adverse effect · Papillary renal cell carcinoma · Vascular endothelial growth factor

Introduction

Renal cell carcinoma (RCC) is the most common kidney tumor, and the incidence has been increasing over the last several decades [1]. Although the most common histological type of RCC is clear cell carcinoma, papillary carcinoma makes up 7–14% of RCCs [2]. Treatment of advanced RCC with cytokines, such as interferon-α and interleukin-2, was the standard practice for a number of years [3]. Although the median survival period of patients with metastatic papillary RCC was reported to be only 5.5 months and no patients survived beyond 2 years [4], a recent study indicated a 5-year survival rate for papillary RCC of 10% [5]. Thus, the prognosis has improved because novel molecular therapeutic agents have demonstrated significant clinical activity in advanced RCC and altered the standard therapy for this disease. Of these therapies, sunitinib, a new oral multitargeted receptor tyrosine kinase inhibitor (TKI), is promising for the treatment of RCC patients. In a phase III trial, progression-free survival was longer, and the response rate was higher in patients with untreated metastatic RCC who received sunitinib than in those receiving interferon-α as conventional therapy [6]. The patients with papillary RCC treated with sunitinib had prolonged median progression-free survival of 11.9 months, although overall clinical responses remained low [7]. On the other hand, this agent shows a broad range of adverse effects 3–4 weeks after initiation of treatment, such as neutropenia, thrombocytopenia, asthenia, hypertension, and bullous skin toxicity. However, pulmonary bleeding as an adverse effect has seldom been reported.

Here, we report a case of metastatic papillary RCC complicated with diffuse alveolar hemorrhage because of sunitinib.

Case report

At the age of 67 years, the patient complained of a non-productive cough in June 2008 and was subsequently hospitalized in July 2008.

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He was a non-smoker. Although he had no family history of cancer, he had a history of tuberculous epididymitis at the age of 20, and was treated with oral therapy for diabetes mellitus and hypertension. The patients' blood cell counts were as follows (normal ranges are shown in parentheses): hemoglobin, 12.1 g/dl (13.5–17.0 g/dl); hematocrit, 36.1% (39.7–51.0%); white blood cells, 7,920/mm^3 (3,300–8,800/mm^3); platelets, 373,000/mm^3 (130,000–350,000/mm^3). The results of renal function, coagulation, and collagen studies, such as myeloperoxidase-anti-neutrophil cytoplasmic antibody (MPO-ANCA), were normal. Whole-body computed tomography (CT) revealed a huge left renal tumor with multiple lung and liver nodules, and mediastinal lymph node swelling. In addition, positron emission tomography (PET) demonstrated accumulating spots in multiple bone tumor lesions. Pathological diagnosis using renal and pulmonary CT-guided needle biopsies showed type 1 papillary RCC with lung metastases. The clinical stage was determined to be T3bN0M1 (primary left renal tumor with lung, bone, liver, and mediastinal lymph node metastases) according to the TNM classification in August 2008.

On admission, his performance status was 1, but he suffered systemic metastases, and the tumor growth progressed rapidly. The tumor doubling time was 42 days. Therefore, he was spared cytoreductive nephrectomy for standard therapy and treated with sunitinib, a novel angiogenic inhibitor, as first-line systemic therapy in September 2008. Daily treatment with sunitinib showed efficacy with progression control in targeting primary and multiple lung and liver metastatic lesions. However, he suffered a small amount of bloody sputum (grade 1; Common Terminology Criteria for Adverse Events ver. 3.0) on day 5 and slight malaise (grade 1) on day 7. Administration of sunitinib was then interrupted because thrombocytopenia (grade 3) developed on day 18. On the same day, the chest CT scan revealed diffuse ground-glass opacity lesions around lung metastatic nodular shadows (Fig. 1a). To investigate the abnormal shadows, flexible bronchoscopy was performed, and serous bloody bronchoalveolar lavage fluid was obtained from the superior lingular segment (Fig. 2). After stopping sunitinib, he was treated with carbazochrome sodium sulfonate hydrate against diffuse alveolar hemorrhage, resulting in a marked reduction of the lesions determined by CT on day 40 (Fig. 1b). Although he was treated with interferon-α after cessation of sunitinib, tumor growth indicated a rapidly progressive course. Then, he received four cycles of sunitinib until diffuse alveolar hemorrhage (grade 2) or thrombocytopenia (grade 3) was observed. In four cycles of sunitinib, controllable diffuse alveolar hemorrhage developed as well as first administration. Finally, the patient died of respiratory failure 8 months after the onset because of the progression of multiple lung and mediastinal lymph node metastases, and pleuritis carcinomatosa and lymphangitic carcinomatosis.

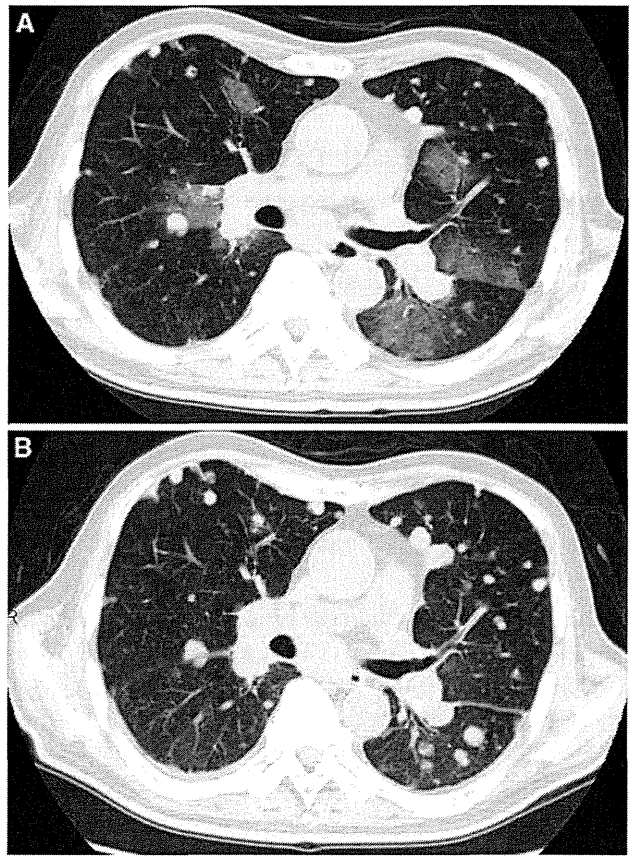

Fig. 1 Chest computed tomography revealing diffuse ground-glass opacity lesions around lung metastases on day 18 after treatment with sunitinib (**a**). The lesions disappeared on day 40 (**b**)

In the lung autopsy, diffuse peripheral alveolar hemorrhage was confirmed around the lung metastases (Fig. 3a). To investigate the mechanism of diffuse alveolar hemorrhage by sunitinib, immunohistochemistry was performed for VEGF in the autopsy specimens of both lung tumors and primary renal tumors in addition to renal biopsy specimens. Although about 60% of tumor cells demonstrated weak cytoplasmic staining for VEGF in the renal biopsy, almost all tumor cells showed strong staining in both lung metastases and primary renal tumor in the autopsy (Fig. 3b).

Discussion

Here we report the first case of diffuse alveolar hemorrhage as an adverse effect of sunitinib in metastatic papillary RCC. Sunitinib is an oral multitargeted TKI against VEGFR-1 and -2, platelet-derived growth factor (PDGF)

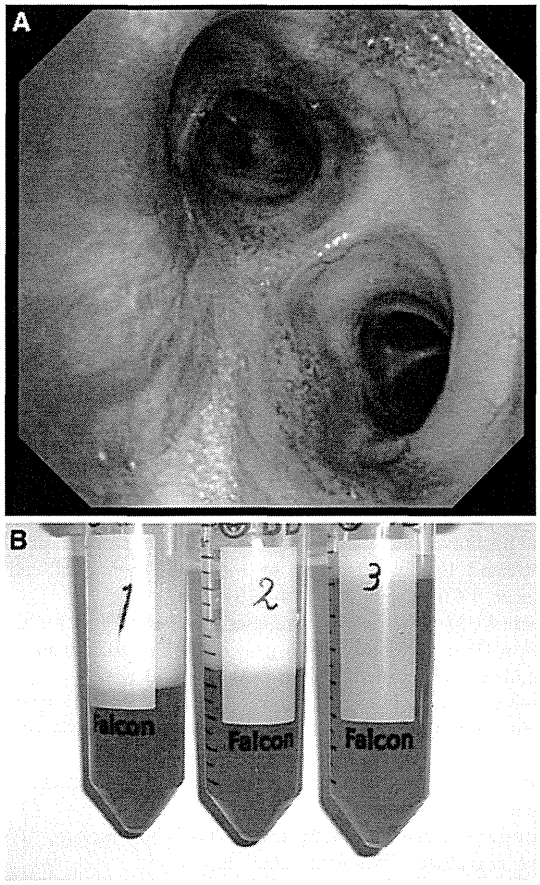

Fig. 2 Flexible bronchoscopy demonstrating bronchial hemorrhage from the superior lingular segment (**a**). Serous blood in bronchoalveolar lavage fluid was observed (**b**)

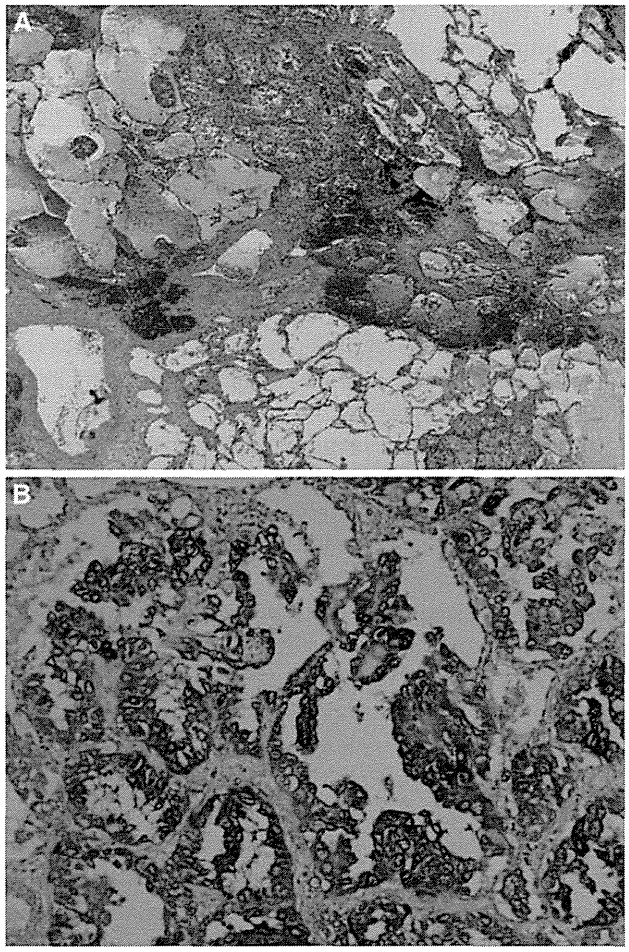

Fig. 3 Histological findings of peripheral alveolar hemorrhage around lung metastases at autopsy (**a**). Immunohistochemical findings showing strongly positive cytoplasmic staining for VEGF in lung metastases at autopsy (**b**). **a** H&E, ×200; **b** immunostaining of VEGF, ×200

receptors α and β, c-KIT, FLIT-3, and RET protooncogene (RET). Substantial clinical activity of sunitinib has been demonstrated in imatinib-resistant gastrointestinal stromal tumors, breast cancer, and other tumors [8]. Similar to other cancers, sunitinib has a major clinical impact for RCC.

Recently, pulmonary bleeding has been reported to be an adverse effect with the development of antiangiogenic therapeutic approaches. Bevacizumab, a recombinant humanized monoclonal antibody that binds to VEGF, showed an adverse effect with fatal tumor-related bleeding, especially centrally located lung tumor or squamous cell histology, in patients enrolled in a randomized study for non-small cell lung carcinoma [9]. Sunitinib led to fatal pulmonary bleeding events in two patients with advanced squamous cell lung cancer in a phase II trial of previously treated advanced non-small cell lung cancer [10]. Therefore, ongoing trials for antiangiogenic agents tend to eliminate patients with squamous cell lung cancer. Furthermore, a retrospective study suggested that baseline tumor cavitation may be a potential risk factor for pulmonary hemorrhage in first-line advanced non-small cell lung cancer treatment with carboplatin and paclitaxel plus bevacizumab [11].

The present case represents a timely warning regarding the clinical use of sunitinib for the following reasons: (1) the histological diagnosis was metastatic lung adenocarcinoma from RCC, but not squamous cell lung cancer, in which sunitinib-related diffuse alveolar bleeding has been reported; (2) lung metastatic tumors with hemorrhage were located in the peripheral side without cavitations; (3) the frequency of the use of sunitinib is anticipated to increase in the future. Therefore, care should be taken with regard to the adverse effects of sunitinib in cases of RCC lung metastases. However, the mechanism and clinical condition of sunitinib-associated pulmonary hemorrhage remain unclear.

VEGF is one of the principal proangiogenic factors in solid tumors and plays a pivotal role in vascular development, stimulating both angiogenesis and vasculogenesis

[12, 13]. Papillary RCC tumors also show high levels of VEGF immunoreactivity [14], and elevated VEGF mRNA levels in papillary RCC tumors were associated with short survival [15]. In addition, this growth factor is known as both a proangiogenic and survival factor for endothelial cells. Inhibition of VEGF results in inhibition of vascular development and significant alteration of epithelial development, suggesting that VEGF coordinates proper development of the normal lung epithelium and vasculature [16]. Therefore, it seems possible that inhibition of VEGF may decrease the regenerative capacity of the endothelial cells, resulting in endothelial dysfunction in the supporting layers of the blood vessels [17].

In the present case, we speculated that the correlation with high-level production of VEGF from the tumor and the anti-VEGF effect of sunitinib in the lung metastatic microenvironment may have caused diffuse alveolar hemorrhage as adverse events because the tumor cells showed stronger expression of VEGF in both the primary site and the lung metastases at autopsy. On the other hand, Kontovinis et al. [18] reported that plasma VEGF-A levels increased after treatment with sunitinib in clear cell metastatic RCC. Therefore, we cannot rule out the possibility that anti-VEGF treatment itself might have induced the increased expression of VEGF in the autopsy specimens. In addition, pulmonary bleeding as an adverse effect of sunitinib has been reported only rarely in papillary RCC tumors. As we did not investigate other sunitinib-targeting molecules, such as PDGF, c-KIT, FLIT-3, and RET, further investigations regarding the mechanism of pulmonary hemorrhage are required.

In conclusion, we reported a case of metastatic papillary RCC with controllable diffuse alveolar hemorrhage as an adverse event associated with sunitinib. Care should be taken in cases of pulmonary hemorrhage with the use of anti-angiogenesis agents not only in squamous cell lung cancer, but also in metastatic lung tumors.

Conflict of interest statement No author has any conflict of interest.

References

1. Chow WH, Devesa SS, Warren JL et al (1999) Rising incidence of renal cell cancer in the United States. JAMA 281:1628–1631
2. Reuter VE (2006) The pathology of renal epithelial neoplasms. Semin Oncol 33:534–543
3. Cohen HT, McGovern FJ (2005) Renal-cell carcinoma. N Engl J Med 353:2477–2490
4. Motzer RJ, Bander NH, Nanus DM (1996) Renal-cell carcinoma. N Engl J Med 335:865–875
5. Patard JJ, Leray E, Rioux-Leclercq N et al (2005) Prognostic value of histologic subtypes in renal cell carcinoma: a multicenter experience. J Clin Oncol 23:2763–2771
6. Motzer RJ, Hutson TE, Tomczak P et al (2007) Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 356:115–124
7. Choueiri TK, Plantade A, Elson P et al (2008) Efficacy of sunitinib and sorafenib in metastatic papillary and chromophobe renal cell carcinoma. J Clin Oncol 26:127–131
8. Socinski MA (2008) The current status and evolving role of sunitinib in non-small cell lung cancer. J Thorac Oncol 3:S119–S123
9. Johnson DH, Fehrenbacher L, Novotny WF et al (2004) Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. J Clin Oncol 22:2184–2191
10. Socinski MA, Novello S, Brahmer JR et al (2008) Multicenter, phase II trial of sunitinib in previously treated, advanced non-small-cell lung cancer. J Clin Oncol 26:650–656
11. Sandler AB, Schiller JH, Gray R et al (2009) Retrospective evaluation of the clinical and radiographic risk factors associated with severe pulmonary hemorrhage in first-line advanced, unresectable non-small-cell lung cancer treated with carboplatin and paclitaxel plus bevacizumab. J Clin Oncol 27:1405–1412
12. Ferrara N, Gerber HP, LeCouter J (2003) The biology of VEGF and its receptors. Nat Med 9:669–676
13. Hanahan D, Weinberg R (2000) The hallmarks of cancer. Cell 100:57–70
14. Jacobsen J, Grankvist K, Rasmuson T et al (2004) Expression of vascular endothelial growth factor protein in human renal cell carcinoma. BJU Int 93:297–302
15. Ljungberg BJ, Jacobsen J, Rudolfsson SH et al (2006) Different vascular endothelial growth factor (VEGF), VEGF-receptor 1 and 2 mRNA expression profiles between clear cell and papillary renal cell carcinoma. BJU Int 98:661–667
16. Zhao L, Wang K, Ferrara N et al (2005) Vascular endothelial growth factor co-ordinates proper development of lung epithelium and vasculature. Mech Dev 122:877–886
17. Cho YJ, Murgu SD, Colt HG (2007) Bronchoscopy for bevacizumab-related hemoptysis. Lung Cancer 56:465–468
18. Kontovinis LF, Papazisis KT, Touplikioti P et al (2009) Sunitinib treatment for patients with clear-cell metastatic renal cell carcinoma: clinical outcomes and plasma angiogenesis markers. BMC Cancer 9:82

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The Role of Percutaneous Needle Biopsy in Differentiation of Renal Tumors

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Objective: The safety and accuracy of active percutaneous needle biopsy for small renal tumors have been reported. However, there have been few reports of passive biopsy for renal tumors without clear pretreatment histological characterization based on imaging studies due to the rarity of these tumors. In this study, we examined the background, accuracy, adverse events and patient prognosis associated with such biopsies.
Methods: Japanese patients with renal tumors histological characteristics of which were unclear on imaging prior to treatment were enrolled in this study and analyzed retrospectively. The study population consisted of 24 renal cell carcinoma patients and 13 non-renal cell carcinoma patients.
Results: Although the percentage of hypervascularity was significantly higher in clear cell renal cell carcinoma compared with the other neoplasms ($P < 0.001$), there were no significant differences between renal cell carcinoma and non-renal cell carcinoma with regard to hypervascularity, hydronephrosis, venous thrombus, hematuria or metastasis. The histological results in eight of nine (89%) nephrectomy patients were in accordance with those of biopsies. The median survival time of all 37 patients was 21 months and the 5-year survival rate was 31.1%. The 5-year survival rates of nephrectomy patients and non-nephrectomy patients were 75 and 0%, respectively. The overall survival of nephrectomy patients was significantly better than that of non-nephrectomy patients ($P = 0.003$).
Conclusions: Biopsy of renal tumors is safe and accurate regardless of the type of guidance and nephrectomy after appropriate diagnosis by biopsy contributed to longer survival.

Key words: needle biopsy – renal tumor – renal cell carcinoma – vascularity – overall survival

INTRODUCTION

The incidence of renal tumors has been increasing in the USA, with 57 760 new cases and 12 980 deaths estimated in 2009 (1). Similarly, the incidence of renal tumors in Japan has also been increasing, with an estimated 4000 deaths from renal tumors in 2007. Renal cell carcinoma (RCC) is the most frequent malignancy of renal tumors and clear cell RCC (cRCC), the most common type of RCC, accounts for 75% of malignant renal tumors (2). Although most cases of RCC are diagnosed as hypervascular, encapsulated tumors with central necrosis by imaging methods, such as computed tomography (CT), differential diagnosis from other types of neoplasm, especially large urothelial carcinoma (UC) occurring in the renal pelvis, is difficult. There are differences between RCC and the other neoplasms with regard to appropriate surgical technique, and in cases with metastasis, the primary site of RCC should be resected. On the other hand, the other neoplasms generally should not be resected and should initially be treated with systemic therapy. Furthermore, the advent of molecular target therapies directed against vascular endothelial growth factor and the mammalian target of rapamycin requires diagnosis of RCC subtype (3–7). Therefore, the histological results of renal

tumors must be predicted before treatment if they cannot be confirmed by imaging. Recently, the safety and accuracy of active percutaneous needle biopsy for small renal tumors have been reported. These reports mainly spotlighted the differentiation between RCC and benign renal tumors due to prevention of unnecessary surgery, because 30% of renal tumors <4 cm in diameter that were removed by radical or partial open nephrectomy were benign on final histological evaluation. However, such concerns are inherent in small renal tumors because the percentage of benign tumors decreased from 46.3% for those <1 cm in diameter to 6.3% for those measuring 7 cm or more (8). There have been few report of passive biopsy for various renal tumors histological characteristics of which were unclear on imaging prior to treatment due to the rarity of these tumors. In the present study, we examined the background, accuracy, adverse events and patient prognosis of such biopsy of renal tumors.

PATIENTS AND METHODS

Patients

Japanese patients with renal tumors without clear pretreatment characterization based on imaging studies were enrolled in this study. All patients had undergone image-guided percutaneous needle biopsy at Kanazawa University Hospital from 1989 to 2009 and they were analyzed retrospectively. All patients were 15 years or older at the time of biopsy. The reasons for the necessity of biopsy were as follows: (i) for differentiation between RCC and UC occurring in the renal pelvis, which could not be diagnosed despite drip infusion pyelography or retrograde pyelography in addition to CT; (ii) for histological identification of inoperable hypervascular tumors with invasion to adjacent organs or multiple metastases; (iii) for histological identification of hypovascular tumors that could not be diagnosed as RCC; (iv) for histological identification of tumors unsuitable for contrast-enhanced CT. CT images of typical cRCC and representative biopsied tumors are shown in Fig. 1. All percutaneous needle biopsies were performed under CT (12 patients) or ultrasound (US) (25 patients) guidance. Before 1996, all biopsies were performed under US guidance, and after 2007, all biopsies were performed under CT guidance. Between 1997 and 2006, as a transition period from US-guided to CT-guided, nine US-guided biopsies and five CT-guided biopsies were performed. All US-guided biopsies were performed by urologists and all CT-guided biopsies were performed by radiologists.

Statistical Analysis

The date of surgery or biopsy was used as the start of observation. Statistical analyses were performed using commercially available software (Prism). Comparisons between two groups were performed by unpaired two-sided *t*-test or Fisher's exact test. The crude probability of survival was estimated using the Kaplan–Meier method. Univariate analysis of differences between patient groups was performed with the log-rank test. Statistical significance was defined as $P < 0.05$.

RESULTS

Patient Population

Thirty-seven patients were included in the present study. Patients were divided into two groups according to the

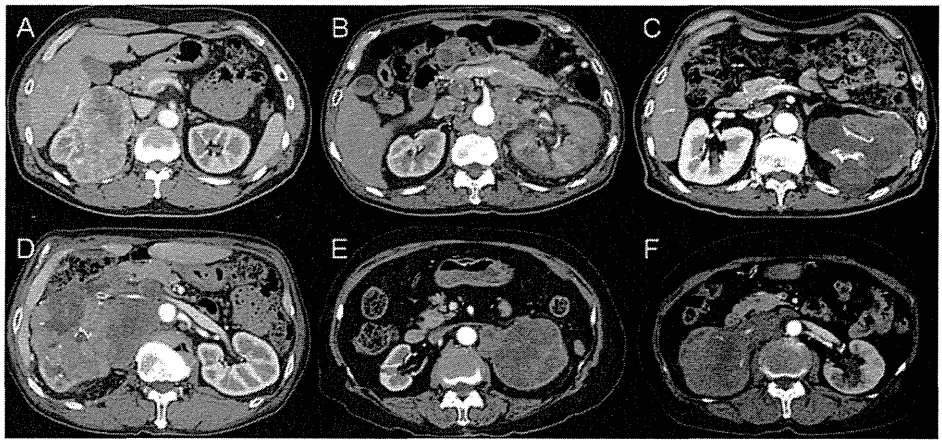

Figure 1. (A) A hypervascular encapsulated tumor with central necrosis consistent with cRCC was not biopsied but treated by surgery and was identified as cRCC. (B) A hypovascular tumor infiltrating the left kidney was identified as cRCC by biopsy. (C) A hypovascular tumor with calcification and hydronephrosis was associated with lung, lymph node (LN) and liver metastases and was identified as pRCC by biopsy. (D) A large hypovascular tumor with adrenal and LN metastases was identified as chromophobe RCC by biopsy. (E) A hypovascular tumor replacing the left kidney was identified as urothelial carcinoma by biopsy. (F) A hypovascular tumor replacing the right kidney with renal venous thrombus was identified as squamous cell carcinoma by biopsy.

histological results: the RCC group and the non-RCC group. Although the histological results diagnosed by biopsy were used, the results of final histological evaluation were used if the patient underwent nephrectomy. Patient demographic and clinical characteristics are shown in Table 1. The numbers of RCC and non-RCC patients were 24 and 13, respectively. The median ages of the patients in the two groups were 61.5 and 69 years, respectively. The median tumor sizes determined by the largest dimension were 8.0 and 9.0 cm, respectively, and the numbers of small renal tumors defined as <4 cm were three and one, respectively. Three patients did not undergo contrast-enhanced CT because of renal impairment, asthma and for unknown reason, respectively. Vascularity was unclear in two patients despite contrast-enhanced CT. Lymph node (LN) metastasis was defined according to the LN classification of RCC and UC, including bilateral renal hilus, abdominal para-aortic, para-inferior vena cava and aortocaval regions. Although we examined whether findings on CT and hematuria allowed prediction of RCC before biopsy or surgery, there were no marked differences between RCC and non-RCC with regard to hypervascularity, hydronephrosis, venous thrombus, hematuria or metastasis. However, the percentage of hypervascularity was significantly higher in cRCC (8 of 11 patients) compared with non-cRCC (2 of 21 patients; $P < 0.001$).

Urine cytology was performed in 17 patients in the RCC group and 11 patients in the non-RCC group. Positive diagnosis was three and one patients, respectively. However, all three positive patients in the RCC group were misdiagnosed as UC and the patient in the non-RCC group was diagnosed as just malignant findings.

Results of Biopsies

The results of biopsies are shown in Table 2. Despite being the most prevalent subtype of RCC, the percentage of cRCC was only 30% in the present study. Various histological types were confirmed. Sufficient tumor sample (only normal tissue) was not extracted for histological examination in 4 of 37 patients. However, the patient with neck LN swelling was diagnosed as having papillary RCC (pRCC) by biopsy of the neck LN, and a diagnosis of angiomyolipoma was made in the patient who underwent plain CT again. Two patients underwent nephrectomy, and were diagnosed as having cRCC and hematoma, respectively. There were no significant differences between the proportions of insufficiency in CT-guided biopsy (no patients) and US-guided biopsy (four patients; $P = 0.2823$).

Biopsies were accompanied by adverse events in four cases; hematuria occurred in two cases and continuous pain or bleeding were observed in one case each. These adverse events resolved spontaneously within 24 h. There were no significant differences between the proportions of adverse events in CT-guided biopsy (no patients) and US-guided

Table 1. Patient demographics and clinical characteristics

	RCC	Non-RCC	*P*
Patients (*n*)	24	13	
Median age (years)	61.5 (15–86)	69 (16–83)	0.651
Sex			
Male	17	5	0.056
Female	7	8	
Affected side			
Right	12	7	0.823
Left	12	6	
Tumor size (cm)			
Median	8.0 (2.0–16.4)	9.0 (3.0–12.8)	0.835
<4 cm	3	1	
>4 cm	21	12	
Vascularity			
Hypervascular	9	1	0.107
Hypovascular	13	9	
Unknown	2	0	
Not enhanced	0	3	
Hydronephrosis			
Yes	5	3	0.874
No	19	10	
Venous thrombus			
Yes	9	3	0.371
No	15	10	
Hematuria			
Yes	7	1	0.441
No	16	11	
Unknown	1	1	
LN meta			
Yes	10	4	0.514
No	14	9	
Distant meta			
Total	17	7	0.301
Lung	11	4	
Bone	6	2	
Distant LN	4	3	
Brain	2	2	
Liver	4	0	
Adrenal	4	0	
Contralateral kidney	1	2	
Peritoneum	0	1	

RCC, renal cell carcinoma; meta, metastasis; LN, lymph node. Data in parentheses are range.

Table 2. Results of biopsies

RCC	
Clear cell type	11
Papillary type	3
Chromophobe type	2
Granular type	1
Sarcomatous type	1
Non-clear cell type	1
Undetermined	2
Urothelial carcinoma	4
Small cell carcinoma	3
Squamous cell carcinoma	1
Non-hodgkin lymphoma	1
Poor differentiated carcinoma	1
Highly malignant tumor	1
Meta of parotid carcinoma	1
Normal tissue (insufficient)	4

RCC, renal cell carcinoma; meta, metastasis.

biopsy (four patients; $P = 0.2823$). There was no tumor seeding along the needle tract during follow-up.

Results of Nephrectomy

Excluding two patients from whom sufficient amounts of tissue could not be extracted for histological examination by biopsy, nine patients underwent nephrectomy according to the results of the biopsy. The final histological results for seven of nine nephrectomy patients were completely in accordance with the histological results of biopsy. One patient diagnosed as having cRCC by biopsy was subsequently confirmed to have pRCC on final histological evaluation. The diagnosis at final histological evaluation (pRCC) was different from the histological results of the biopsy (UC) in only one patient.

Overall Survival

The median survival time of all 37 patients was 21 months and the 5-year survival rate was 31.1% (Fig. 2A). The overall survival of patients who underwent nephrectomy was significantly better than that of patients who did not undergo nephrectomy ($P = 0.003$). The 5-year survival rates of nephrectomy patients and non-nephrectomy patients were 75 and 0%, respectively. The median survival time of non-nephrectomy patients was 8 months (Fig. 2B). The median ages of nephrectomy patients and of non-nephrectomy patients were 55 years (range: 15–65 years) and 68.5 years (range: 16–86 years), respectively ($P = 0.034$). The median tumor sizes in nephrectomy patients and non-nephrectomy patients were 8 cm (range: 2–16.4 cm) and 8.75 cm (range: 2.5–16.2 cm), respectively ($P = 0.380$). The numbers of nephrectomy patients and non-nephrectomy patients with either LN or distant metastasis were 3 and 24, respectively ($P = 0.001$).

DISCUSSION

Renal tumor biopsy has become common in the evaluation of small renal tumors because imaging alone is insufficient to show the underlying aggressiveness of these lesions and more ablative therapies are available (9). On the other hand, the role of conventional percutaneous biopsy for renal tumors has been limited, and there is little general experience to date (10). In the present study, we evaluated the background, accuracy, adverse events and patient prognosis of such passive biopsy. The median tumor size was 8.3 cm and the percentage of small renal tumors defined as <4 cm was only 11%. To our knowledge, the median tumor size in the present study is the largest reported. The percentage of cases with metastases was 65%. Accordingly, renal tumors that had to be biopsied were generally progressive. In addition, a wide range of histological results were found and the percentage of cRCC, the most common type of RCC, was only 30% in the present study, although it was 74% in a recent report (11). Hydronephrosis, which is inherent in UC, and venous thrombus, which is inherent in RCC, did not

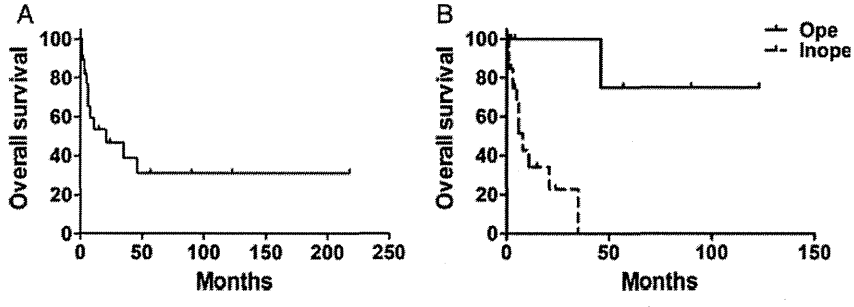

Figure 2. (A) Kaplan–Meier analysis of the overall survival in all 37 biopsied patients. (B) Kaplan–Meier analysis of the overall survival in nephrectomy and non-nephrectomy patients. The solid line shows the overall survival of nephrectomy patients excluding two patients with insufficient biopsy samples ($n = 9$). The broken line shows the overall survival of non-nephrectomy patients ($n = 26$).

predict UC and RCC as histological results, and hypervascularity alone strongly predicted cRCC and may allow the patient to avoid undergoing biopsy.

Biopsy was non-diagnostic in 4 of 37 patients (11%) in the present study. The reported percentage of non-diagnostic biopsies was 0–21% (12–15). Although histological results in eight of nine nephrectomy tumors (89%) were in accordance with histological results of biopsies, the subtype of RCC was different between the histological results of biopsy and nephrectomy in one patient. It was reported that the percentage accuracy was 86.7–100% (12–15). The most controversial potential complication of renal tumor biopsy is the risk of tumor seeding along the needle tract. However, the overall estimated risk is $<0.01\%$ and only six cases of seeding have been reported (10). In consideration of these results, our data were equivalent to the standard outcome regardless of type of guidance used.

Importantly, the overall survival of patients who underwent nephrectomy was significantly better than that of patients who did not undergo nephrectomy. There have been no previous reports regarding the overall survival focused on nephrectomy after biopsy. Although there was no significant difference between the median tumor size of nephrectomy patients and non-nephrectomy patients, the median age of the former was significantly younger than that of the latter and the percentage of non-nephrectomy patients who had either LN or distant metastasis was significantly higher than that of nephrectomy patients. Biopsy may effectively extract patients with metastasis who should undergo nephrectomy, such as those with RCC, and exclude non-RCC patients with metastasis, lymphoma and small cell carcinoma which are very sensitive to chemotherapy from candidates for nephrectomy.

The present study had a number of limitations. Histological grade was not considered as a prognostic variable because it was not clear in some specimens and the success of grading by percutaneous needle biopsy is controversial (16). Needles used were mostly 18 gauges with a tissue cutting tip; however, precise records were not available. Wunderlich et al. (17) recommended obtaining one central and one peripheral biopsy specimen from tumors smaller than 4 cm and two peripheral biopsies from larger tumors. Although at least two peripheral biopsies were obtained in all CT-guided biopsies, the precise numbers of US-guided biopsies were not available in our series. In addition, all patients were Japanese, so the distribution of RCC according to histological subtype or the percentage of RCC in renal tumors may differ in patients from other ethnic backgrounds. More importantly, this study was retrospectively analyzed with small sample size in a single institute. It may have prevented determination of the precise statistical significance. To avoid unnecessary nephrectomy of a kidney with hypovascular tumor, the usefulness of prior biopsy should be validated. Long followed-up and larger prospective studies comparing prior biopsy group to upfront nephrectomy group without biopsy in patients with hypovascular renal tumor may be needed to confirm our findings.

Finally, there were various histological results in renal tumors without clear pretreatment characterization based on imaging studies and hypervascularity may be an important predictive factor of cRCC. It was confirmed that biopsies of such tumors were safe and accurate regardless of the type of guidance used. Nephrectomy after diagnosis by biopsy might contribute to longer survival.

CONCLUSIONS

The patients who underwent biopsy for renal tumors without clear pretreatment histological characterization based on imaging studies tended to show progressive disease. Owing to the wide variety of histological results, hypovascular tumors should be biopsied actively before treatment. It was confirmed that biopsy of such tumors was safe and accurate regardless of the type of guidance used. Nephrectomy after diagnosis by biopsy might contribute to longer survival.

Conflict of interest statement

None declared.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009;59:225–49.
2. Atkins MB, Choueiri TK, Cho D, Regan M, Signoretti S. Treatment selection for patients with metastatic renal cell carcinoma. *Cancer* 2009;115:2327–33.
3. Rini BI, Halabi S, Rosenberg JE, Stadler WM, Vaena DA, Ou SS, et al. Bevacizumab plus interferon alfa compared with interferon alfa monotherapy in patients with metastatic renal cell carcinoma: CALGB 90206. *J Clin Oncol* 2008;26:5422–8.
4. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Staehler M, et al. Sorafenib for treatment of renal cell carcinoma: Final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial. *J Clin Oncol* 2009;27:3312–8.
5. Hudes G, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, et al. Global ARCC Trial. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *N Engl J Med* 2007;356:2271–81.
6. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, et al., RECORD-1 Study Group. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. *Lancet* 2008;372:449–56.
7. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Oudard S, et al. Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2009;27:3584–90.
8. Frank I, Blute ML, Cheville JC, Lohse CM, Weaver AL, Zincke H. Solid renal tumors: an analysis of pathological features related to tumor size. *J Urol* 2003;170:2217–20.
9. Remzi M, Marberger M. Renal tumor biopsies for evaluation of small renal tumors: why, in whom, and how? *Eur Urol* 2009;55:359–67.
10. Volpe A, Kachura JR, Geddie WR, Evans AJ, Gharajeh A, Saravanan A, et al. Techniques, safety and accuracy of sampling of renal tumors by fine needle aspiration and core biopsy. *J Urol* 2007;178:379–86.
11. Sofikerim M, Tatlisen A, Canoz O, Tokat F, Demirtas A, Mavili E. What is the role of percutaneous needle core biopsy in diagnosis of renal masses. *Urology*; Epub ahead of print 26 January 2010.
12. Hara I, Miyake H, Hara S, Arakawa S, Hanioka K, Kamidono S. Role of percutaneous image-guided biopsy in the evaluation of renal masses. *Urol Int* 2001;67:199–201.

13. Lechevallier E, Andre M, Barriol D, Daniel L, Eghazarian C, De Fromont M, et al. Fine-needle percutaneous biopsy of renal masses with helical CT guidance. *Radiology* 2000;216:506–10.
14. Caoili EM, Bude RO, Higgins EJ, Hoff DL, Nghiem HV. Evaluation of sonographically guided percutaneous core biopsy of renal masses. *AJR Am J Roentgenol* 2002;179:373–8.
15. Shah RB, Bakshi N, Hafez KS, Wood DP, Jr, Kunju LP. Image-guided biopsy in the evaluation of renal mass lesions in contemporary urological practice: indications, adequacy, clinical impact, and limitations of the pathological diagnosis. *Hum Pathol* 2005;36: 1309–15.
16. Neuzillet Y, Lechevallier E, Andre M, Daniel L, Coulange C. Accuracy and clinical role of fine needle percutaneous biopsy with computerized tomography guidance of small (less than 4.0 cm) renal masses. *J Urol* 2004;171:1802–5.
17. Wunderlich H, Hindermann W, Al Mustafa AM, Reichelt O, Junker K, Schubert J. The accuracy of 250 fine needle biopsies of renal tumors. *J Urol* 2005;174:44–6.

ORIGINAL

Epidemiological and clinical features of lung cancer patients from 1999 to 2009 in Tokushima Prefecture of Japan

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Abstract : Lung cancer is the leading cause of malignancy-related death worldwide. In the present study, we reviewed the epidemiologic and clinical features of lung cancer in Tokushima Prefecture, Japan. Between January 1999 and December 2009, 2,183 patients with lung cancer were enrolled in this study. One thousand five hundred ninety-one (73%) patients were male and 592 (27%) patients were female. Median age was 70 years, with a range of 15-93 years. Seventy-six percent of patients had smoking history. One thousand nine hundred five (87%) patients were non-small cell lung cancer and the predominant histological type was adenocarcinoma (51%). Among all 2,183 patients, 702 (32%) belonged to elderly population. Four hundred seventy-one (22%), 213 (10%), 24 (1%), 116 (5%), 238 (11%), 370 (17%) and 678 (31%) patients had stage IA, IB, IIA, IIB, IIIA, IIIB and IV lung cancer, respectively. In Tokushima University Hospital, 516 (29%), 191 (11%), 58 (3%), 755 (43%) and 216 (12%) patients were initially treated with chemotherapy, chemo-radiotherapy, thoracic radiotherapy, operation and best supportive care, respectively. The median time to progression (TTP) and the median survival time (MST) of patients treated with chemotherapy and chemo-radiotherapy were 3.5 months, 13.0 months and 7.0 months, 18.0 months, respectively. The median TTP and the MST of 33 elderly patients treated with chemotherapy were 3.3 months and 18.0 months, respectively, which were comparable with those of total population. These results indicated the benefit of chemotherapy in elderly patients with advanced lung cancer by proper selection. J. Med. Invest. 57 : 326-333, August, 2010

Keywords **:** *epidemiology, lung cancer, Tokushima Prefecture*

Abbreviation used : TTP, time to progression ; OS, overall survival ; NSCLC, non-small cell lung cancer ; SCLC, small cell lung cancer ; MST, median survival time ; RR, response rate.

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INTRODUCTION

Lung cancer is a global public health problem of epidemic proportions, and the number of people affected is expected to grow in the near future (1). Despite improvements in survival for many other types of cancer in recent years, 5-year survival for lung cancer has remained relatively poor, mainly because by the time a diagnosis is made, lung cancer is frequently advanced and treatment options are limited (2-4).

An estimated 1.35 million people were newly diagnosed with lung cancer worldwide in 2002 (12.4% of all new cancers) (5), an increase of about 110,000 compared with the number in 2000 (6). In addition, lung cancer is the leading cause of malignancy-related death worldwide (5). In 2002 there were about 1.18 million deaths caused by lung cancer internationally (5), an increase of over 70,000 deaths since 2000 (6). Lung cancer deaths caused almost 18% of total cancer mortality (5, 7), and around 2% of all mortality worldwide during 2002 (7).

Lung cancer is, to a major extent, a disease of the elderly (8). The prevalence and societal burden of this disease will increase as more people survive into old age. Elderly patients with cancer are significantly under-represented in all clinical trials, including in those for lung cancer (9-11). A retrospective analysis of all patients enrolled onto Southwest Oncology Group trials between 1993 and 1996 demonstrated that only 25% were 65 years or older, whereas this age subgroup made up 63% of the U.S. population of patients with cancer (11). The low enrollment of patients older than 70 years was largely responsible for this discrepancy (11).

In Japan, 62,063 (45,189 male and 16,874 female) patients died of lung cancer, which consisted of 19% of all malignancy-related death in 2005, and more than half of them (53%) belonged to 75 years or older, so-called elderly patient population (12). Tokushima Prefecture, a regional area located in southeast part of Shikoku Island, Japan, had 459 (342 male and 117 female) patients who died of lung cancer in 2005, and inclined to have more elderly patients (58%) than all parts of Japan (12).

While the outlines of epidemiology of lung cancer have been reported as mentioned above, detailed epidemiologic and clinical trends in Tokushima Prefecture still remain uncertain. In the present study, we reviewed the epidemiology of lung cancer in Tokushima Prefecture focusing on 1) the incidence by age and histology, and 2) stage, treatment modalities and clinical outcome in comparison between elderly patient and younger or total populations.

PATIENTS AND METHODS

Patient eligibility

The patients who had been either cytologically or histologically confirmed to have lung cancer in Tokushima University Hospital and Tokushima Prefectural Central Hospital from 1999 to 2009 were eligible for this retrospective study. Tokushima University Hospital and Tokushima Prefectural Central Hospital are two main hospitals engaging in lung cancer treatment in Tokushima Prefecture and more than 50% of lung cancer patients in Tokushima Prefecture were treated in these two hospitals. Therefore, we considered that patient population in these two hospitals was able to recapitulate the epidemiology of lung cancer in Tokushima Prefecture. We defined 74 years or younger patients as younger population and 75 years or older patients as elderly population. The study protocol was approved by the Institutional Review Board of each of the participating institution.

Evaluation of response and toxicity for treatment

Enrolled patients were appropriately treated with standard treatment modalities for lung cancer depending on their general status. Chest X-ray, complete blood count, and blood chemistry studies were repeated at least once a month for follow-up. The response was assessed based on the computed tomography scan findings that initially had been used to define the tumor extent. The response was evaluated in accordance with the Response Evaluation Criteria in Solid Tumors version 1.0.

Statistical analysis

For the evaluation of the efficacy of chemotherapy, we investigated the time to progression (TTP) and overall survival (OS) of lung cancer patients who received any chemotherapeutic agents in Tokushima University Hospital. The TTP was defined as the time from diagnosis to progression or death from any cause. The OS was defined as the time from diagnosis to death from any cause or when last known to be alive. The TTP and the OS were estimated by the Kaplan-Meier method of univariate analysis. The differences between categorized groups were compared by the One-way ANOVA test. All statistical