Figure 2   Localization of androgen receptors in prostate cancer cell lines. Immunocytochemical staining of prostate cancer cell lines, LNCaP, DU145, and PC-3, and COS-1 transfected with the expression plasmid of AR (COS-1) was performed with anti-AR antibody. Immunofluorescence staining for AR was performed with LNCaP cells (LNCaP IF). Upper: cells were cultured in the absence of ligand. Lower: cells were cultured in the presence of 10^{-6} M testosterone. Bar = 10 μm. ·

gens, whereas in LNCaP cells, AR-GFP was detected not only in the cytoplasm but also in the nucleus without nucleoli. The primary localization of AR-GFP in LNCaP cells was similar to the localization of endogenous AR. Upon the addition of testosterone, AR-GFP in all cell lines was time-dependently translocated into the nucleus and showed a discrete pattern that formed subnuclear foci. It took <20 min to form nuclear foci in all investigated cells after treatment with testosterone. Intranuclear foci were distributed evenly throughout the nucleus without nucleoli. Intensity of foci formation was not changed after overnight treatment with the ligand (data not shown). No significant difference in the localization and trafficking pattern of the AR-GFP was detected between LNCaP cells (androgen sensitive) and DU 145/PC-3 cells (androgen refractory).

Subcellular Localization and Trafficking of AR (T877A)-GFP With High Concentration of Androgen

We transfected plasmids expressing AR (T877A)-GFP to COS-1 and LNCaP cells (Figure 4). In both cells, the primary localization of AR (T877A)-GFP was not only in the cytoplasm but also in the nucleus in the absence of testosterone. However, a predominance of AR-GFP was observed in the nucleus. Upon the addition of 10^{-6} M testosterone, AR (T877A)-GFP in both cell lines was time-dependently translocated into the nucleus and formed subnuclear foci. Trafficking patterns and distribution in the nucleus of both cells were the same as those observed in the case of wild-type AR-GFP.

Trafficking Pattern of AR-GFP and AR (T877A)-GFP With Low Concentration of Androgen

We also transfected plasmids expressing AR-GFP and AR (T877A)-GFP to COS-1 and LNCaP cells and observed the effect of a low concentration of androgen (Figure 5). With 10^{-9} M DHT treatment, the distribution of AR-GFP and AR (T877A)-GFP in COS-1 cells remained cytoplasmic, whereas those in LNCaP cells were translocated to the nucleus and formed subnuclear foci, showing the same pattern as in the high androgen concentration with regard to the time course, distributional pattern, and their size.

Trafficking Pattern of AR-GFP and AR (T877A)-GFP With Antiandrogen Treatment

We used bicalutamide (CAS), a competitive androgen antagonist, as a ligand at 10^{-5} M, almost the same as the plasma concentration of the clinical dose (Figure 6). Upon the addition of CAS, AR-GFP that was transfected in both COS-1 and LNCaP cells was translocated into the nucleus but did not form subnuclear foci, whereas AR (T877A)-GFP in both cells was translocated to the nucleus and formed typical subnuclear foci. The distribution and trafficking pattern of AR (T877A)-GFP in LNCaP cells and COS-1 cells were the same as those observed in the case of AR-GFP.

Discussion

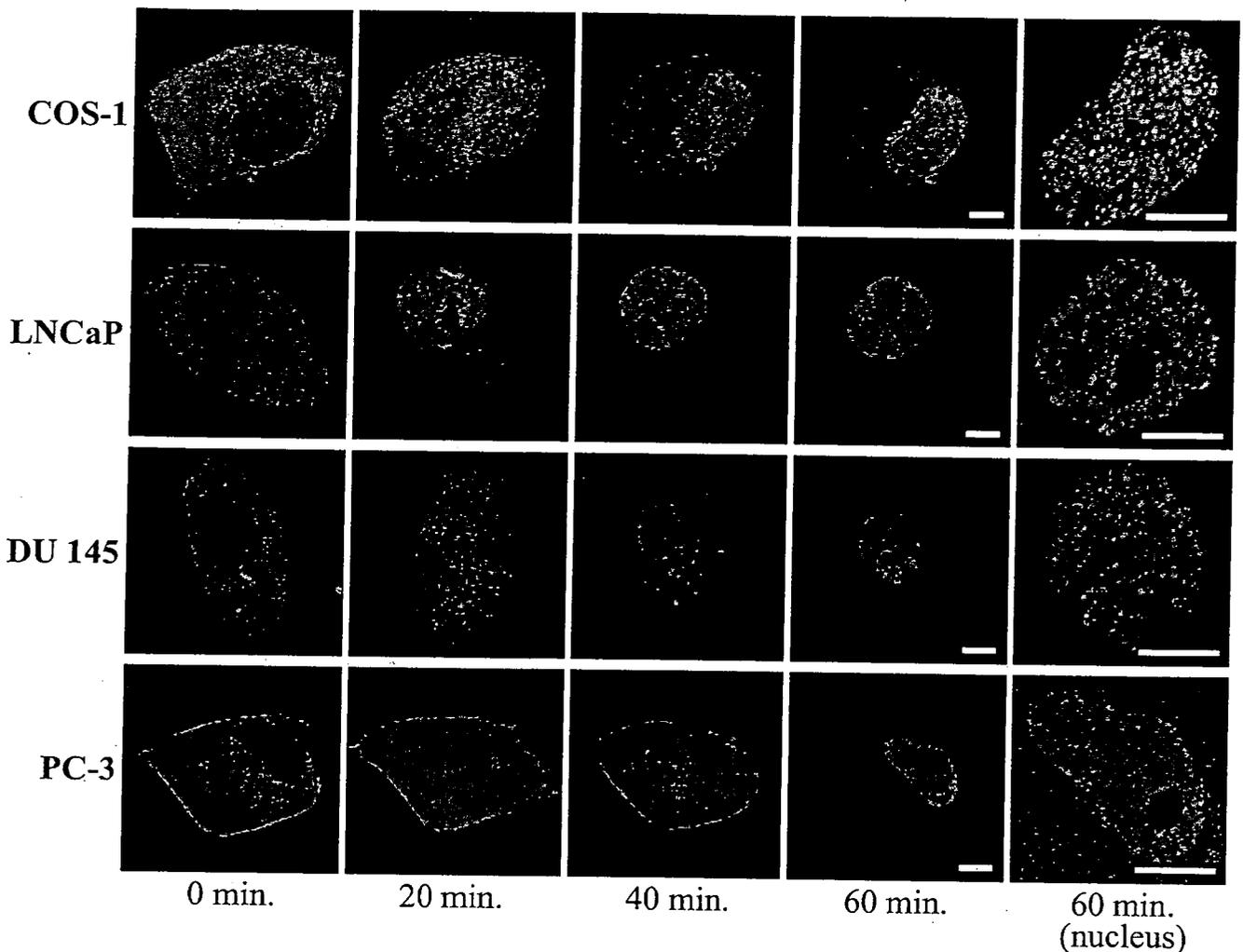The present study using a real-time imaging method clearly showed that wild-type AR-GFP and mutated

Figure 3 Real-time imaging of AR-green fluorescent protein (AR-GFP) in prostate cancer cell lines and COS-1 cells treated with 10^{-6} M testosterone. COS-1, LNCaP, DU 145, and PC-3 cells transfected with the expression plasmid of AR-GFP were cultured in the absence of ligand for 14 hr before observation. Fluorescent images were captured by confocal laser scanning microscopy every 20 min after the addition of 10^{-6} M testosterone. Bar = 5 μm.

AR (T877A)-GFP in prostate cancer cells, LNCaP, and non-prostate cancer cells, COS-1, translocated to their nucleus and formed subnuclear foci at a high concentration of androgen, whereas a low concentration of androgen led to AR-GFP and AR (T877A)-GFP forming nuclear foci only in the LNCaP cells and not in the COS-1 cells. In addition, it was demonstrated that anti-androgen treatment induced subnuclear foci in LNCaP and COS-1 cells that were transfected with AR (T877A)-GFP, but not in those with AR-GFP (Table 1).

AR activation by androgen is a multistep process. AR in the cytoplasm forms a complex with chaperone proteins such as heat-shock protein 90 (HSP90), HSP70, and HSP56 (Picard and Yamamoto 1987; Hager et al. 2000; Pratt et al. 2004). Ligand binding leads to a conformational change of its receptor followed by revealing the NLS (Simental et al. 1991; Zhou et al. 1994; Gelmann

2002). Many studies have suggested that exposed NLS of the cytoplasmic cargo protein is recognized by the importin family that mediates translocation to the nucleus through the nuclear complex, and the direct binding of Ran GTP to importin–cargo complex releases the cargo into the nucleoplasm (Picard and Yamamoto 1987; Poukka et al. 2000). There has been, however, no direct evidence of an interaction between AR and the importin family. Further studies are needed to elucidate the subcellular compartmentalization of AR.

With the use of the three-dimensional construction of confocal microscopic images with AR-GFP, it was demonstrated that after binding with the agonist, AR-GFP is concentrated in a subnuclear compartment that has the appearance of foci in a boundary region of the nucleus between euchromatin and heterochromatin (Tomura et al. 2001). The functional significance of these
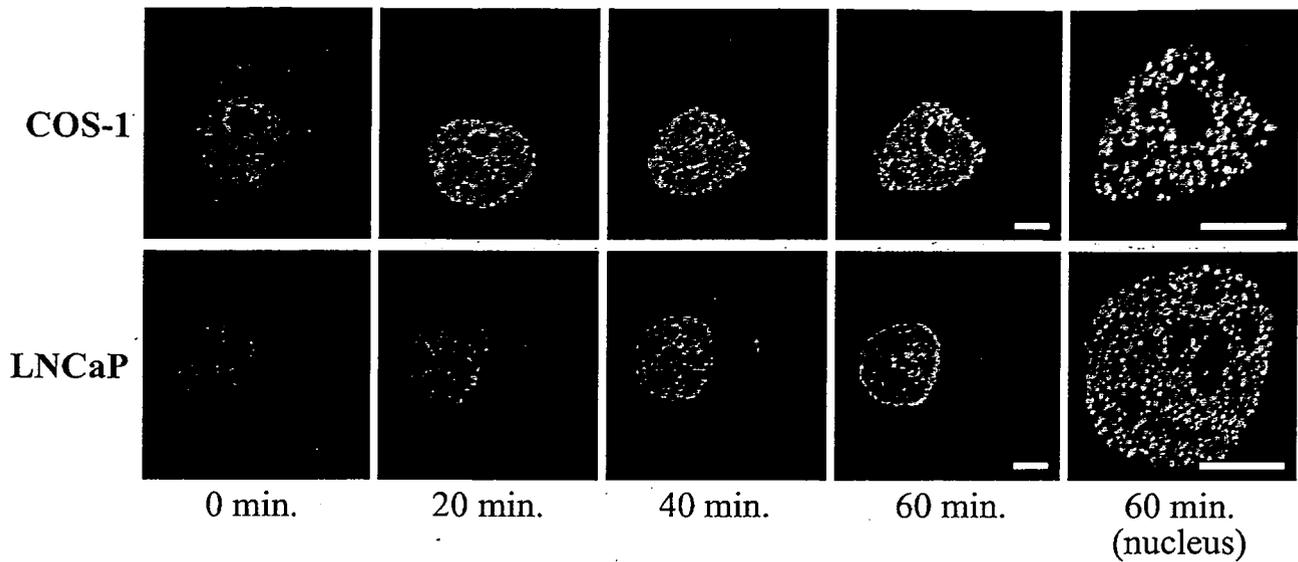
COS-1

LNCaP

0 min.   20 min.   40 min.   60 min.   60 min.
                                                                    (nucleus)

Figure 4   Real-time imaging of AR (T877A)-GFP in COS-1 and LNCaP cells treated with 10⁻⁶ M testosterone. COS-1 and LNCaP cells transfected with the expression plasmid of AR (T877A)-GFP were cultured in the absence of ligand for 14 hr before observation. Fluorescent images were captured by confocal laser scanning microscopy every 20 min after the addition of 10^{-6} M testosterone. Bar = 5 μm.

subnuclear foci has been discussed at the molecular level. The interaction of AR with this compartment is strongly biased for the agonist-bound form of AR (Tomura et al. 2001; Karvonen et al. 2002; Black et al. 2004). Several studies have shown that the interaction between AR and SRC1, GRIP-1, and the p160 coactivator family in these subnuclear foci is an agonist-dependent re-cruitment and/or release step that occurs before AR engages with chromatin (Karvonen et al. 2002; Black et al. 2004).

Most prostate cancer patients initially respond to androgen ablation therapy (Huggins and Hodges 1941); therefore, it is considered that androgen plays pivotal roles in the growth promotion of epithelial tissue of prostate cancer. The LH-RH analog excludes testicular androgen production, and antiandrogen excludes adrenal androgens. This combination of the two medicines is the first-line hormone therapy of prostate cancer. However, the disease eventually progresses to what is defined as hormone-refractory prostate cancer (HRPC), at which point the tumor is no longer responsive to androgen ablation, and uncontrolled progression of the disease is inevitable (Palmberg et al. 1999). Several possible mechanisms have been proposed (Grossmann et al. 2001). Mutations in the ligand-binding domain of the AR gene or amplification of the AR gene would increase AR sensitivity to a low concentration of androgen or allow it to respond to other steroids or anti-androgens. Many mutation sites of the AR gene in prostate cancer cells have been reported (Gottlieb et al. 2004), and codon 877 mutation (threonine to alanine) is the most frequently detected. This mutation is also

found in LNCaP cell lines (Veldscholte et al. 1990). It was previously reported that mutations of the AR gene result in a higher affinity to androgen (Tilley et al. 1996). Moreover, it is an accepted theory that this results in the broadening ligand specificity of AR (Gaddipati et al. 1994; Taplin et al. 1995; Suzuki et al. 1996). Codon 877 of AR is located in the LBD and contacts the ligand directly. Mutation at this site alters the stereochemistry of helix 11 that forms part of the ligand-binding pocket. The present study showed that antiandrogen treatment did not induce the formation of subnuclear foci of AR-GFP in the COS-1 and LNCaP cells but apparently produced those foci of AR (T877A)-GFP in COS-1 and LNCaP cells. However, Farla et al. (2005) demonstrated that AR (T877A)-GFP did not form foci in CAS-treated Hep3B cells. These results indicate that T877A mutation broadens the specificity of ligand recognition (McDonald et al. 2000), but subnuclear foci formation of antiandrogen-bound AR (T877A) appears to depend on the intracellular environment, e.g., levels of interacting regulatory factors in the cellular milieu.

The present study demonstrated that AR-GFP and AR (T877A)-GFP in LNCaP cells were translocated to the nucleus and formed subnuclear foci at both low and high concentrations of androgen, and that those in COS-1 cells were translocated at the high concentration but not at the low concentration. These results suggest that T877A mutation of the AR gene does not account for AR hypersensitivity. It is supposed that hypersensitivity of AR might be due to the existence of some other intracellular factors such as chaperone proteins, signaling cascade, and cofactors in LNCap cells. Some
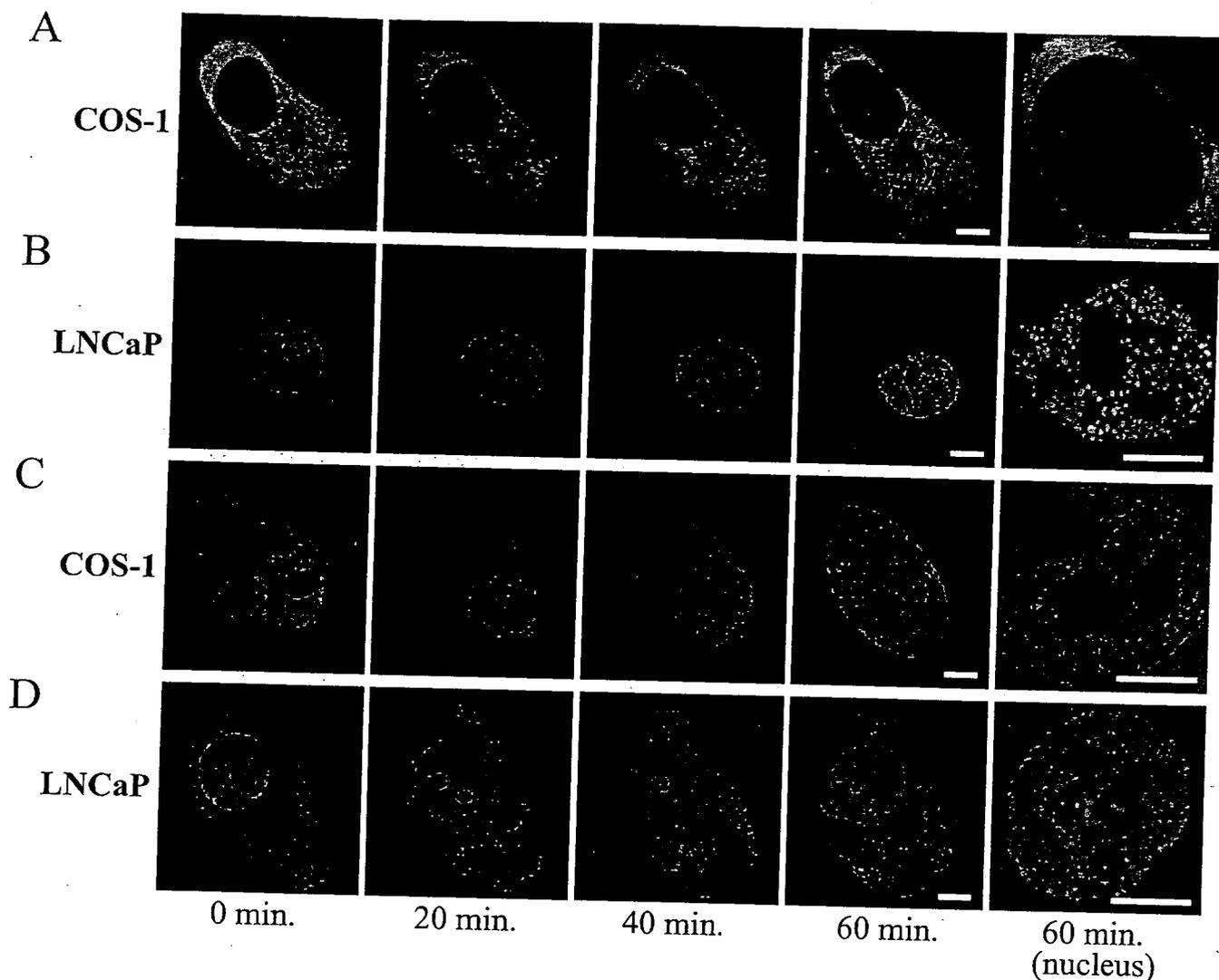
Figure 5 Real-time imaging of AR-GFP and AR (T877A)-GFP in COS-1 and LNCaP cells treated with 10^{-9} M dihydrotestosterone (DHT). COS-1 (A,C) and LNCaP (B,D) cells transfected with the expression plasmid of AR-GFP (A,B) or AR (T877A)-GFP (C,D) were cultured in the absence of ligand for 14 hr before observation. Fluorescent images were captured by confocal laser scanning microscopy every 20 min after the addition of 10^{-9} M DHT. Bar = 5 μm.

cofactors, for example, the 160-kDa nuclear receptor coactivator (p160) family, interact directly with AF-1 of AR and can also bind to LBD (Ma et al. 1999). Consequently, they enhance the AR-mediated transcription of target genes in a ligand-dependent manner (Alen et al. 1999). One possibility is that an increase of the p160 family, such as glucocorticoid receptor-interacting protein 1 (GRIP-1) and the steroid receptor coactivator-1 (SRC1), may allow low concentrations of androgen to function more efficiently as an AR ligand. However, this is still controversial because expression levels of SRC1 protein in HRPC cells varied in experiments (Gregory et al. 2001; Linja et al. 2004).

The fusion protein with AR was detected in the cytoplasm without androgen treatment in non-prostate cells; however, AR-GFP in LNCaP cells localizes not only in the cytoplasm but also in the nucleus. Subcellular factors in prostate cancer may activate the AR-signaling pathway itself or another one besides AR, having an interdependence on AR. Another possibility is that prostate cancer may have specific docking proteins for substrate binding in a nuclear import pathway. Furthermore, AR (T877A)-GFP in non-prostate cancer cells was detected in both the cytoplasm and nucleus. Alterations in the AR gene may lead to AR having an affinity for the nucleus.

The presence of endogenous AR in prostate cancer cells has been controversial (Tilley et al. 1995). The present study clearly showed that AR protein was not expressed in DU 145 or PC-3 cells, but was expressed in LNCaP cells at the same level of expression as in COS-1 cells transfected with rAR by Western blot analysis using anti-AR antibody. Immunocytochemistry by the
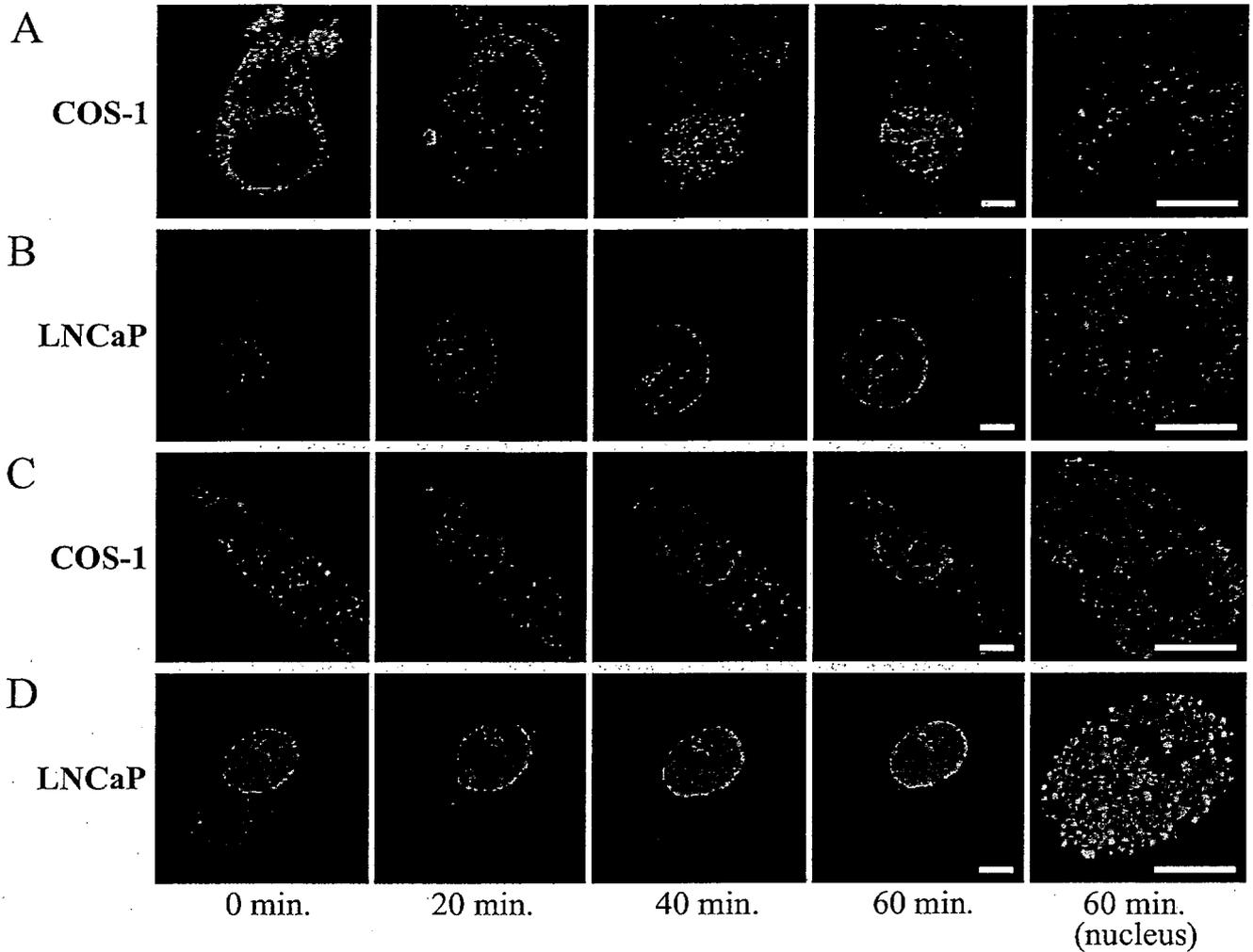
Figure 6   Real-time imaging of AR-GFP and AR (T877A)-GFP in COS-1 and LNCaP cells treated with 10^{-5} M bicalutamide (CAS). COS-1 (A,C) and LNCaP (B,D) cells transfected with the expression plasmid of AR-GFP (A,B) or AR (T877A)-GFP (C,D) were cultured in the absence of ligand for 14 hr before observation. Fluorescent images were captured by confocal laser scanning microscopy every 20 min after the addition of 10^{-5} M CAS. Bar = 5 μm.

streptavidin–biotin peroxidase method with anti-AR antibody was consistent with Western blot analysis, showing that AR immunoreactivity was observed only in LNCaP cells and not in DU 145 or PC-3 cells. A recent report demonstrated the expression of AR protein in DU 145 and PC-3 cells (Alimirah et al. 2006). This contradictory result might be due to the different antibodies used.

The concepts of compartmentalization of AR in the cell and nuclear import of AR to the nucleus in response to androgen and antagonist have extended our understanding of the molecular mechanisms of prostate cancer progression. The present study of live imaging using GFP may facilitate further study on the relationship between AR and prostate cancer.

Table 1   Summary of differential response of AR and AR (T877A)

	AR-GFP 10^{-6} M T	AR (T877A)-GFP 10^{-6} M T	AR-GFP 10^{-9} M DHT	AR (T877A)-GFP 10^{-9} M DHT	AR-GFP 10^{-5} M CAS	AR (T877A)-GFP 10^{-5} M CAS
COS-1	+(*)	+(*)	−	−	+	+(*)
LNCaP	+(*)	+(*)	+(*)	+(*)	+	+(*)

−, no change; +, translocation without subnuclear foci; +(*), translocation with subnuclear foci.
AR-GFP, androgen receptor–green fluorescent protein; DHT, dihydrotestosterone; CAS, bicalutamide.

Literature Cited

Alen P, Claessens F, Verhoeven G, Rombauts W, Peeters B (1999) The androgen receptor amino-terminal domain plays a key role in p160 coactivator-stimulated gene transcription. Mol Cell Biol 19:6085–6097

Alimirah F, Chen J, Basrawala Z, Xin H, Choubey D (2006) DU-145 and PC-3 human prostate cancer cell lines express androgen receptor: implications for the androgen receptor functions and regulation. FEBS Lett 580:2294–2300

Black BE, Paschal BM (2004) Intranuclear organization and function of the androgen receptor. Trends Endocrinol Metab 15:411–417

Black BE, Vitto MJ, Gioeli D, Spencer A, Afshar N, Conaway MR, Weber MJ, et al. (2004) Transient, ligand-dependent arrest of the androgen receptor in subnuclear foci alters phosphorylation and coactivator interactions. Mol Endocrinol 18:834–850

Chang CS, Kokontis J, Liao ST (1985) Structural analysis of complementary DNA and amino acid sequences of human and rat androgen receptors. Proc Natl Acad Sci USA 85:7211–7215

Eisenfeld AJ (1975) Characteristics of steroid hormone receptors in brain and pituitary. In Stumpf WE, Grant LD, eds. Anatomical Neuroendocrinology. Basel, Karger, 52–61

Farla P, Hersmus R, Trapman J, Houtsmuller AB (2005) Antiandrogens prevent stable DNA-binding of the androgen receptor. J Cell Sci 118:4187–4198

Gaddipati JP, McLeod DG, Heidenberg HB, Sesterhenn IA, Finger MJ, Moul JW, Srivastava S (1994) Frequent detection of codon 877 mutation in the androgen receptor gene in advanced prostate cancers. Cancer Res 54:2861–2864

Gelmann EP (2002) Molecular biology of the androgen receptor. J Clin Oncol 20:3001–3015

Georget V, Lobaccaro JM, Terouanne B, Mangeat P, Nicolas JC, Sultan C (1997) Trafficking of the androgen receptor in living cells with fused green fluorescent protein-androgen receptor. Mol Cell Endocrinol 129:17–26

Gottlieb B, Beitel LK, Wu JH, Trifiro M (2004) The androgen receptor gene mutations database (ARDB): 2004 update. Hum Mutat 23:527–533

Gregory CW, He B, Johnson RT, Ford OH, Mohler JL, French FS, Wilson EM (2001) A mechanism for androgen receptor-mediated prostate cancer recurrence after androgen deprivation therapy. Cancer Res 61:4315–4319

Grossmann ME, Huang H, Tindall DJ (2001) Androgen receptor signaling in androgen-refractory prostate cancer. J Natl Cancer Inst 93:1687–1697

Hager GL, Lim CS, Elbi C, Baumann CT (2000) Trafficking of nuclear receptors in living cells. J Steroid Biochem Mol Biol 74:249–254

Heinlein CA, Chang C (2004) Androgen receptor in prostate cancer. Endocr Rev 25:276–308

Huggins C, Hodges CV (1941) Studies on prostatic cancer: the effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res 1:293–297

Karvonen U, Janne OA, Palvimo JJ (2002) Pure antiandrogens disrupt the recruitment of coactivator GRIP1 to colocalize with androgen receptor in nuclei. FEBS Lett 523:43–47

Kawata M, Matsuda K, Nishi M, Ogawa H, Ochiai I (2001) Intracellular dynamics of steroid hormone receptor. Neurosci Res 40:197–203

Kumar S, Saradhi M, Chaturvedi NK, Tyagi RK (2006) Intracellular localization and nucleocytoplasmic trafficking of steroid receptors: an overview. Mol Cell Endocrinol 246:147–156

Linja MJ, Porkka KP, Kang Z, Savinainen KJ, Janne OA, Tammela TL, Vessella RL, et al. (2004) Expression of androgen receptor coregulators in prostate cancer. Clin Cancer Res 10:1032–1040

Lu S, McKenna SE, Cologer-Clifford A, Nau EA, Simm NG (1998) Androgen receptor in mouse brain: sex differences and similarities in autoregulation. Endocrinology 139:1594–1601

Ma H, Hong H, Huang SM, Irvine RA, Webb P, Kushner PJ, Coetzee GA, et al. (1999) Multiple signal input and output domains of the 160-kilodalton nuclear receptor coactivator proteins. Mol Cell Biol 19:6164–6173

MacLean HE, Warne GL, Zajac JD (1997) Localization of functional domains in the androgen receptor. J Steroid Biochem Mol Biol 62:233–242

McDonald S, Brive L, Agus DB, Scher HI, Ely KR (2000) Ligand responsiveness in human prostate cancer: structural analysis of mutant androgen receptors from LNCaP and CWR22 tumors. Cancer Res 60:2317–2322

Nordeen SK, Housley PR, Wan Y, Day RN (2001) Application of green fluorescent protein to the study of dynamic protein-protein interactions and subcellular trafficking of steroid receptors. Methods Mol Biol 176:179–199

Ochiai I, Matsuda K, Nishi M, Ozawa H, Kawata M (2004) Imaging analysis of subcellular correlation of androgen receptor and estrogen receptor α in single living cells using green fluorescent protein color variants. Mol Endocrinol 18:26–42

Palmberg C, Koivisto P, Visakorpi T, Tammela TL (1999) PSA decline is an independent prognostic marker in hormonally treated prostate cancer. Eur Urol 36:191–196

Picard D, Yamamoto KR (1987) Two signals mediate hormone-dependent nuclear localization of the glucocorticoid receptor. EMBO J 6:3333–3340

Poukka H, Karvonen U, Janne OA, Palvimo JJ (2000) Covalent modification of the androgen receptor by small ubiquitin-like modifier 1 (SUMO-1). Proc Natl Acad Sci USA 97:14145–14150

Pratt WB, Galigniana MD, Morishima Y, Murphy PJ (2004) Role of molecular chaperones in steroid receptor action. Essays Biochem 40:41–58

Press MF, Greene GL (1988) Immunocytochemical localization estrogen progesterone receptors. In DeLellis R, ed. Advances in Immunohistochemistry. New York, Raven Press, 341–361

Prins GS, Birch L, Greene GL (1991) Androgen receptor localization in different cell types of the adult rat prostate. Endocrinology 129:3187–3199

Shimomura O, Johnson FH, Saiga Y (1962) Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. J Cell Comp Physiol 59:223–239

Simental JA, Sar M, Lane MV, French FS, Wilson EM (1991) Transcriptional activation and nuclear targeting signals of the human androgen receptor. J Biol Chem 266:510–518

Simental JA, Sar M, Wilson EM (1992) Domain functions of the androgen receptor. J Steroid Biochem Mol Biol 43:37–41

Stumpf WE (1983) The histochemistry of steroid hormone "receptors". J Histochem Cytochem 31:113–114

Stumpf WE, Roth LJ (1966) High resolution autoradiography with dry mounted, freeze-dried frozen sections: comparative study of six methods using two diffusible compounds ^{3}H-estradiol and ^{3}H-mesobilirubinogen. J Histochem Cytochem 14:274–287

Suzuki H, Akakura K, Komiya A, Aida S, Akimoto S, Shimazaki J (1996) Codon 877 mutation in the androgen receptor gene in advanced prostate cancer: relation to antiandrogen withdrawal syndrome. Prostate 29:153–158

Taplin ME, Bubley GJ, Shuster TD, Frantz ME, Spooner AE, Ogata GK, Keer HN, et al. (1995) Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. N Engl J Med 332:1393–1398

Tenbaum S, Baniahmad A (1997) Nuclear receptors: structure, function and involvement in disease. Int J Biochem Cell Biol 29:1325–1341

Tilley WD, Bentel JM, Aspinall JO, Hall RE, Horsfall DJ (1995) Evidence for a novel mechanism of androgen resistance in the human prostate cancer cell line, PC-3. Steroids 60:180–186

Tilley WD, Buchanan G, Hickey TE, Bentel JM (1996) Mutations in

the androgen receptor gene are associated with progression of human prostate cancer to androgen independence. Clin Cancer Res 2:277–285

Tomura A, Goto K, Morinaga H, Nomura M, Okabe T, Yanase T, Takayanagi R, et al. (2001) The subnuclear three-dimensional image analysis of androgen receptor fused to green fluorescence protein. J Biol Chem 276:28395–28401

Tyagi RK, Lavrovsky Y, Ahn SC, Song CS, Chatterjee B, Roy AK (2000) Dynamics of intracellular movement and nucleocytoplasmic recycling of the ligand-activated androgen receptor in living cells. Mol Endocrinol 14:1162–1174

Veldscholte J, Ris-Stalpers C, Kuiper GG, Jenster G, Berrevoets C, Claassen E, van Rooij HC, et al. (1990) A mutation in the ligand binding domain of the androgen receptor of human LNCaP

cells affects steroid binding characteristics and response to antiandrogens. Biochem Biophys Res Commun 173:534–540

Walsh PJ, Teasdale J, Cowen PN (1990) Ultrastructural localisation of oestrogen receptor in breast cancer cell nuclei. Histochemistry 95:205–207

Yeh S, Chang HC, Miyamoto H, Takatera H, Rahman M, Kang HY, Thin TH, et al. (1999) Differential induction of the androgen receptor transcriptional activity by selective androgen receptor coactivators. Keio J Med 48:87–92

Zhou ZX, Sar M, Simental JA, Lane MV, Wilson EM (1994) A ligand-dependent bipartite nuclear targeting signal in the human androgen receptor: requirement for the DNA-binding domain and modulation by NH2-terminal and carboxyl-terminal sequences. J Biol Chem 269:13115–13123

informa
healthcare

ORIGINAL ARTICLE
Preclinical Therapeutics

The Growth-Inhibitory Effects of Dexamethasone on Renal Cell Carcinoma *In Vivo* and *In Vitro*

Yasuyuki Arai,[1] Norio Nonomura,[1] Yasutomo Nakai,[1] Kazuo Nishimura,[1] Daizo Oka,[1] Masahiro Shiba,[1]
Masashi Nakayama,[1] Hitoshi Takayama,[1] Yoichi Mizutani,[2] Tsuneharu Miki,[2] and Akihiko Okuyama[1]

Department of Urology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan.[1]
Department of Urology, Kyoto Prefectural University of Medicine, Kyoto, Japan.[2]

ABSTRACT

Background: Recently, several kinase inhibitors have been reported to exert stronger growth inhibitory effects on metastatic renal cell carcinomas (RCCs) than cytokines such as interferons (IFNs) and interleukin-2 (IL-2). On the contrary, the adverse effects of these drugs are also severe. The aim of this study is to analyze the growth-inhibitory effects of DEXamethasone (DEX) on RCC *in vivo* and *in vitro*. *Methods*: The MTT assay was performed using three RCC cell lines, OUR-10, Caki-1, and NC65. OUR-10 cells were subcutaneously transplanted to the dorsal area of nude mice. The nuclear translocation of glucocorticoid receptor (GR) and NF-κB was examined using appropriate antibodies. Concentrations of interleukin-6 (IL-6), IL-8, and vascular endothelial cell growth factor (VEGF) in the conditioned media and cytosol were measured by enzyme-linked immunosorbent assay (ELISA). *Results*: All three RCC cell lines responded to DEX treatment. The growth of OUR-10 xenografts was significantly inhibited by administration of DEX. GR was translocated into the nucleus on DEX treatment. Intracellular IL-6, as well as IL-6 in the conditioned medium, decreased in OUR-10 cells following treatment with increasing amounts of DEX. Concentrations of IL-8 and VEGF in the conditioned medium of OUR-10 and NC65 cells also decreased following DEX treatment, with the inhibition of nuclear translocation of NF-κB. *Conclusion*: DEX treatment is a candidate for advanced RCC therapy by inhibiting the activation of NF-κB and its downstream products such as IL-6, IL-8 and VEGF.

INTRODUCTION

Renal cell carcinoma accounts annually for more than 30,000 new cases of cancer and more than 12,000 deaths in the United States of America. According to the data from the Surveillance, Epidemiology, and End Results Registry

http://www.seer.cancer.gov), it is localized in 53% of cases, regionally advanced in 20%, and distant in 22%: the corresponding 5-year survival rates are 90%, 61%, and 10%, respectively. Despite the important stage-related risk of tumor progression, until now no effective adjuvant treatment for use after surgery has been established (1). Immunotherapy with interferon (IFN) alfa and interleukin-2 (IL2), improve survival of patients with advanced RCC with only a limited period (2–5). Very recently, inhibitors against several kinases have improved survival periods. However, they were accompanied by severe adverse effects (6).

IL-6 is an inflammatory cytokine that has been reported to stimulate the growth of renal cancer (7, 8). Dexamethasone (DEX) has long been used to suppress the inflammatory reactions often seen in patients with advanced cancers; it acts through activation of transcription factor NF-κB (9, 10). In this study, we have analyzed the growth inhibitory effects of DEX on renal cancer cells *in vivo* and *in vitro* in terms of suppression of NF-κB activation.

METHODS

Cell lines

The human RCC cell lines Caki-1 and the human prostate cancer cell line DU145 were obtained from the American Type Culture Collection (Manassas, Virginia, USA). OUR-10 cell line was established in our laboratory (11). Cell line NC65 was obtained from the Department of Urology, Kyoto Prefectural University of Medicine. OUR-10, NC-65 and Caki-1 cells were maintained in RPMI-1640 containing 10% heat-inactivated fetal calf serum (FCS). DU145 cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% heat-inactivated FCS.

Cell growth assay

To assess cell growth, OUR-10, NC-65 and Caki-1 (1.5×10^3 cells/well) cells were seeded in a 96-well culture plate in RPMI containing 5% charcoal-stripped FCS. Medium containing DEX (10^{-10} to 10^{-6} M) or an equivalent volume of ethanol (0.1%) was added on the next day (day 1) and 2 days later (day 3). On day 6, cell viability was measured with the MTT assay kit (Chemicon International, Temecula, California, USA) according to the manufacturer's instructions. Cell growth was calculated relative to the mean cell growth of control cells, defined as 1.0.

Preparation of cellular protein and western blotting

RCC cells (5×10^5 cells) were seeded onto 100 mm dish plates. On the next day, the medium was changed to RPMI containing 5% charcoal-stripped FCS containing each specific dose of DEX. After 48 h of incubation, the cells were washed twice with phosphate-buffered saline (PBS), collected, suspended in PBS and centrifuged (10000 g, 10 min, 4°C). Cell pellets were homogenized in RIPA buffer (1 × PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 10 μg/mL phenylmethylsulfonyl fluoride, 5 μg/mL aprotinin, and 1:100 dilution of Phosphatase Inhibitor Cocktail 2 (Sigma, St. Louis, Missouri, USA). Supernatant was used as protein lysate. After determination of protein concentration, the supernatant was diluted in the gel loading buffer and boiled for 3 min. Aliquots of each sample corresponding to 10 μg protein were analyzed by 7.5% SDS-PAGE on a polyacrylamide gel. Standard protein markers were used for molecular weight calibration. After blotting, the membrane was blocked with 5% skim milk, washed with PBS containing 0.1% Tween 20 (T-PBS), and probed with specific primary antibody (1:1000 dilution) for 1 h. After another wash in T-PBS, the membrane was incubated for 1 h with the secondary antibody (1:1000 dilution). After a further wash in T-PBS, immunoreactive bands were visualized for horseradish peroxidase with the enhanced chemiluminescence (ECL) substrate according to the manufacturer's instructions (Amersham Pharmacia Biotech, Tokyo, Japan).

In vivo xenograft model

Animal care was in accordance with the laboratory animal guidelines of the Institute of Experimental Animal Sciences in Osaka University Medical School. To establish OUR-10 tumors in mice, OUR-10 cells were detached form dishes with trypsin (0.02%) and resuspended in RPMI with 10% FCS. Six week old male athymic nude mice (n = 6) were given a single subcutaneous injection of 1×10^7 cells in 0.1 mL RPMI in the dorsal area. Three times a week, 6 mice were given a subcutaneous injection of DEX (1 μg per mouse per injection) that had been dissolved in ethanol and diluted 1:2000 in sterile saline immediately before injection; DEX treatment was started at the time of cell inoculation. Six control mice were given an injection of ethanol diluted 1:2000 in sterile saline. The tumor volumes were measured weekly with a slide caliper and determined by the following generally used formula: (length × width^2)/2. When the experiments were completed, the mice were sacrificed.

Immunofluorescence and immunohistochemistry

Cells were treated with 10^{-7} M DEX or ethanol control for 48 h then fixed with methanol. After blocking with 3% bovine serum albumin (BSA), cells were incubated overnight at 4°C with anti-NF-κB (Santa Cruz Biotechnology, Inc., Santa Cruz, California, USA) at a dilution of 1:100 in PBS. Cells were incubated at room temperature for 1 h with a secondary antibody conjugated with FITC (Santa Cruz Biotechnology, Inc.) at a dilution of 1:100, and then treated with the SlowFade Light Antifade Kit with DAPI (Molecular Probes Inc., Eugene, Oregon, USA). To examine GR staining in tissue sections, OUR-10 xenograft tumors were fixed in 10% neutral buffered formalin, processed and embedded in paraffin. Serial tissue sections (5 μm thick) were obtained and mounted on slides that were then deparaffinized, rehydrated, and incubated with 3% (v/v) hydrogen peroxide in PBS for 15 min at room temperature to inhibit endogenous peroxidase activity. The sections were then blocked with 10% BSA for 15 min at 37°C and incubated with rabbit polyclonal anti-GR antibody (Santa Cruz Biotechnology, Inc.) at a dilution of 1:100 in PBS for 4 h at 37°C. The sections were then rinsed three times with PBS, incubated with a peroxidase-conjugated anti-rabbit IgG (Bio-Red Laboratories, Richmond, California, USA) at a dilution of 1:1000 in PBS for 10 min at 37°C, and exposed to avidin-biotin-peroxidase complex using DAKO kit (DAKO Corp., Carpinteria, California, USA) according to the manufacturer's instructions. The sections were reacted with 3.3'-diaminobenzidine as the chromogen and counterstained with hematoxylin. An isotype-specific rabbit IgG was used instead of the GR antibody to serve as a negative control.

Measurement of IL-6, IL-8 and VEGF

To determine the amount of IL-6, IL-8 or VEGF being produced by the RCC cell lines, ELISAs were performed. RCC cells were seeded and the medium was changed to RPMI containing 5% FCS with or without DEX. Conditioned medium was collected 48 h after DEX addition and subjected to ELISA
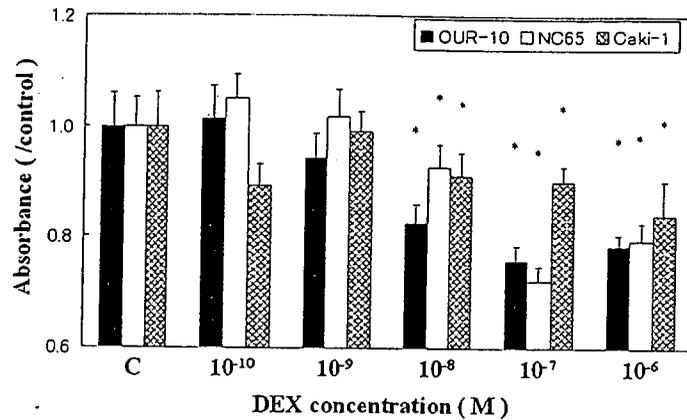
Y. Arai et al.

Figure 1. Effect of DEX on the proliferation of RCC cell lines. Each cell line was cultured in the presence of DEX (10^{-10} to 10^{-6} M), or 0.1% ethanol as control, for 6 days. Each data point represents the mean (n = 6) ± 95% confidence interval (error bars). *p < 0.05.

assay using ELISA kits (Amersham Pharmacia Biotech, Tokyo, Japan) according to the manufacturer's instructions. To measure the intracellular concentration of IL-6, cells were lysed by freeze/thaw (5 cycles) in PBS. After centrifugation (14000 g × 10 min, 4°C), the supernatant was subjected to ELISA assay as above.

Statistical analysis

The level of statistical significance was calculated by the Mann-Whitney U test, assuming statistical significance as p < 0.05. All statistical analyses used the StatView software (SAS Institute Inc., Cary, North Carolina, USA). All statistical tests were two-sided.

RESULTS

Growth-inhibitory effects of DEX on RCC cell lines

RCC cell lines were treated with DEX at concentrations of 10^{-6}–10^{-10} M for 6 days. MTT assays revealed that DEX at concentrations of 10^{-6}–10^{-8} M inhibited growth of OUR-10, NC65 and Caki-1 cells (Figure 1).

GR expression and its downregulation in RCC cells

GR protein expression was examined by Western blot. OUR-10 and NC65 cells expressed high levels of the GR protein, while Caki-1 expressed a low level (Figure 2A). In OUR-10 and NC65 cells, the highest concentration of DEX (10^{-6} M) restored cell growth slightly. In these cell lines, GR protein expression was downregulated *in vitro* by increasing concentrations of DEX (Figure 2B). In order to examine the inhibition of NF-κB activation by DEX, immunofluorescence staining of NF-κB in OUR-10 cells were treated with 10^{-7} M DEX for 48 h. The nuclear expression of NF-κB was attenuated in DEX-treated cells compared with control cells (Figure 2C).
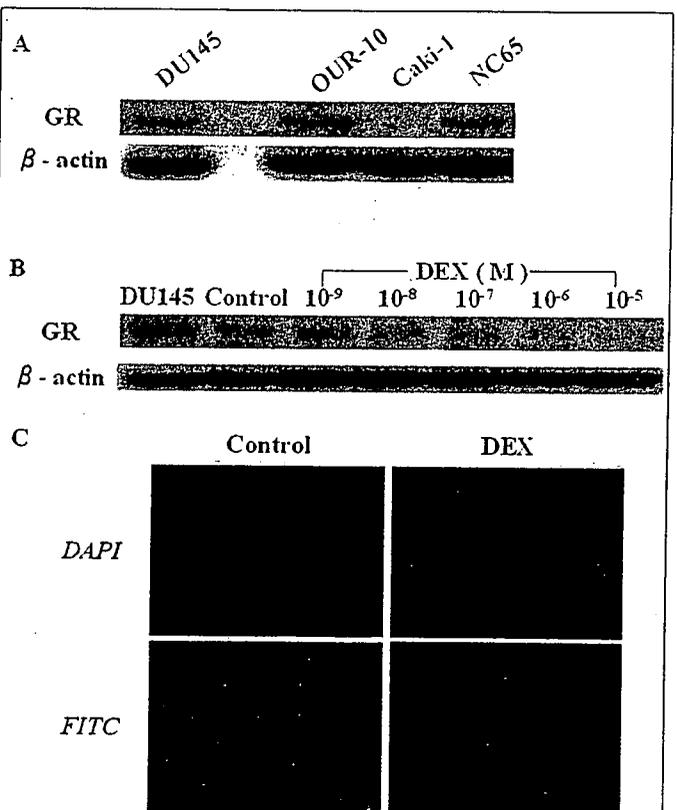

Figure 2. The expression of GR in renal cancer cell lines. **A:** GR expression by Western Blot analysis in RCC cell lines. DU145 prostate cancer cell line is included as a positive control. **B:** Down regulation of GR in OUR-10 cells with increasing concentrations of DEX. OUR-10 cells were treated with increasing concentrations of DEX (10^{-9} to 10^{-5} M) or 0.1% ethanol as control for 48 h. Whole-cell lysates were prepared, and GR protein levels determined by Western blot analysis. **C:** Distribution of GR in OUR-10 cells with or without DEX treatment. OUR-10 cells were incubated with NF-κB p65 antibody, followed by a FITC-conjugated antibody to rabbit IgG, and then counterstained with DAPI. Immunofluorescence staining of OUR-10 cells treated with DEX (10^{-7} M) for 48 hours showed more attenuated translocation of NF-κB p65 from the cytoplasm to the nucleus than control-treated OUR-10 cells.
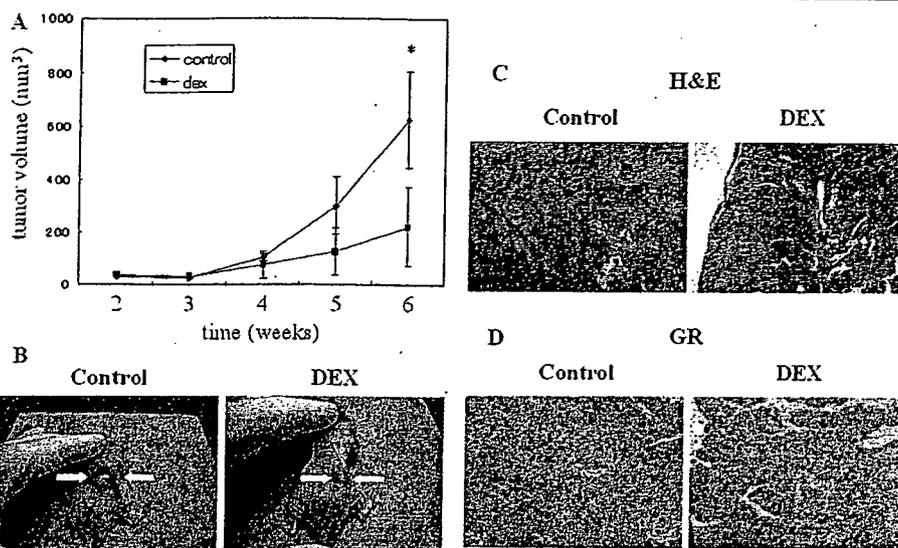
Figure 3. Effect of dexamethasone on growth of OUR-10 cells *in vivo*. **A:** Nude mice were given a subcutaneous injection of OUR-10 cells in the dorsal area. The mice were then injected subcutaneously with low-dose DEX or ethanol (control) for 6 weeks. Arrows identify the location of the tumors. **B:** The volume of each tumor was measured at weekly intervals. Each data point represents the mean (n = 6) ± 95% confidence interval (error bars). *p < 0.05. **C:** Hematoxylin and eosin (H + E) staining of xenografts at 5 week. **D:** GR immunohistochemistry on OUR-10 xenografts.

Growth-inhibitory effects of DEX on OUR-10 xenografts

We examined whether DEX could inhibit the growth of OUR-10 cells *in vivo*. We determined the dose and frequency of low-dose DEX treatment as 1 μg per mouse 3 times per week. After 6 weeks, the mean tumor volume in the DEX-treated mice was 220.9 mm^3 (95% CI = 267 to 399), statistically significantly smaller (p = 0.01) than in the ethanol-treated control mice (629.5 mm^3; 95% CI = 651 to 811) (Figure 3 A, B).

GR expression in OUR-10 xenografts

To determine whether *in vivo* administration of low-dose DEX alters the level of GR expression, we harvested the OUR-10 xenografts from the nude mice after 6 weeks of low-dose treatment. H&E staining showed more inflammatory cells and necrotic tissues in the control group than the DEX groups (Figure 3C). Immunohistochemically, GR was stained mainly I the cytoplasm in xenograft tumors of the ethanol-treated control group, while it was located mainly in the nucleus in the tumors of the DEX-treated group (Figure 3D).

Effects of DEX on IL-6 secretion and intracellular IL-6 concentration

The IL-6 concentration in the cell culture medium of the four RCC cell lines treated with the increasing concentrations of DEX (10^{-10}–10^{-6} M) for 48 h was quantified using ELISA. DEX downregulated IL-6 secretion to less than 50% in OUR-10, NC65 and Caki-1 cells (Figure 4A). Intracellular IL-6 concentrations of the four RCC cell lines treated with DEX (10^{-10}–10^{-6} M) for 48 h were quantified using ELISA and found to be downregulated by approximately 40–70% in all cell lines (Figure 4B).

Effects of DEX on IL-8 and VEGF secretion by RCC cell lines

Concentrations of IL-8 and VEGF in the cell culture media of the four RCC cell lines treated with DEX (10^{-10}–10^{-6} M) for 48 h were quantified using ELISA kits. DEX downregulated IL-8 secretion remarkably in NC65 cells and a little in OUR-10 cells (Figure 4C), and downregulated VEGF secretions by more than 30–65% in all RCC cell lines (Figure 4D).

DISCUSSION

RCC expresses several steroid hormone receptors including the estrogen receptor, progesterone receptor and GR. However, hormone therapy using estrogen or progesterone has not proved useful in RCC (12).

The growth-inhibitory effects of DEX on multiple myeloma and hormone-refractory prostate cancer have been reported (13-15). In the past, DEX has only been shown to inhibit the growth of RCC *in vitro* (16). However, in this study, we demonstrated the growth-inhibitory effects of DEX on three RCC cell lines *in vitro* and *in vivo*. In the experiments to examine dose-dependence, the highest concentration (10^{-6} M) of DEX did not inhibit the growth of RCC cells. Western blot analysis of the GR suggested that this decrease in growth inhibition appeared to be due to GR downregulation caused by increasing concentrations of DEX. These phenomena have been also reported on hormone-refractory prostate cancer (HRPC) (14). The growth-inhibitory effects of DEX on RCC were observed at concentrations
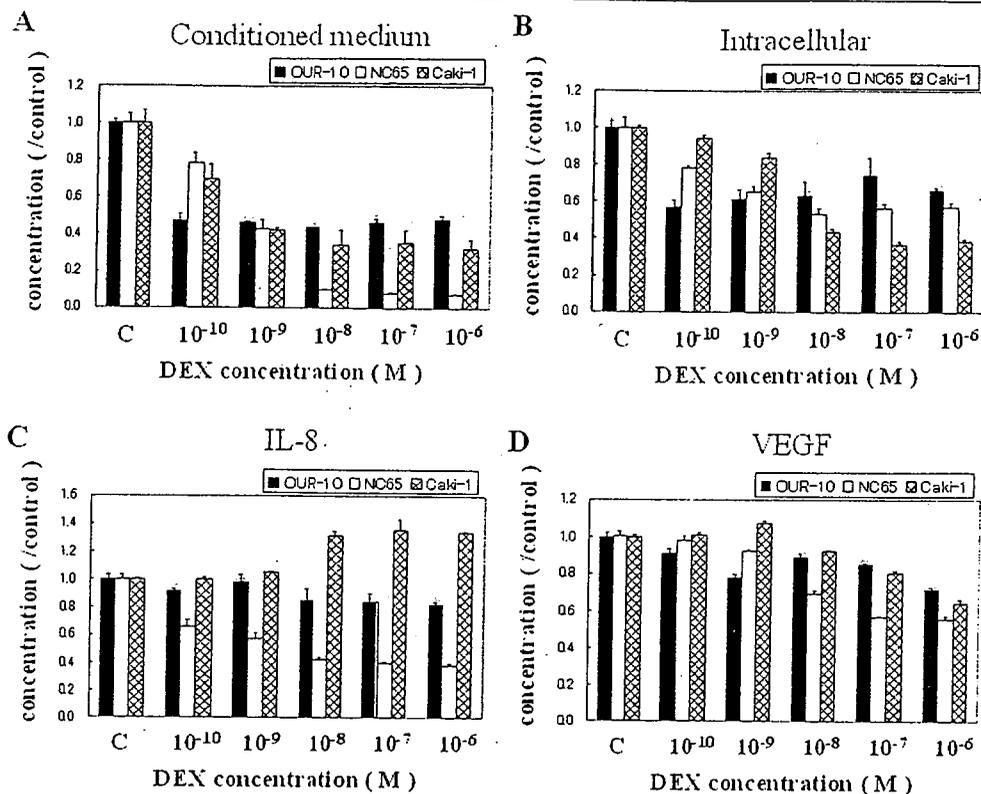
Figure 4. Decrease in IL6, IL8 and VEGF by OUR-10 cells with DEX treatment. **A, B:** IL-6 concentration in conditioned medium (A) and whole cell extract (B) was measured by ELISA assay kits. RCC cell lines were treated with DEX (10^{-10} to 10^{-6} M) or 0.1% ethanol as control for 48 hours. **C, D:** IL-8 (C) and VEGF (D) concentrations in the conditioned medium of each RCC cell lines treated with DEX (10^{-10} to 10^{-6} M) or 0.1% ethanol as control for 48 hours were measured by ELISA.

of 10^{-8}–10^{-7} M, which are physiological concentrations for humans.

In HRPC, DEX inhibits growth by inhibiting the activation of NF-κB. We hypothesized that the same mechanism of growth-inhibition might apply in RCC. IL-6, IL-8 and VEGF are the target genes of NF-κB, and, as shown in our *in vitro* experiments, IL-6 concentrations in the RCC cell line conditioned media correlate with DEX dose. Moreover, the concentration of IL-6 in the cell lysate from RCC cell lines also decreased on DEX administration. IL-6 has been reported to promote the growth of RCC in a paracrine or intracrine fashion (7, 17, 18) and is well known to cause paraneoplastic syndrome (8, 18). In end-stage malignancies, we often encounter an elevated serum IL-6 level. Therefore, in the clinical field, DEX is thought to be useful for patients with end-stage malignancies as palliative therapy because it suppresses those paraneoplastic symptoms due to the elevated IL-6 through inhibiting the NF-κB activation. In Caki-1 cells, concentrations of IL-6 in the conditioned medium and cell lysates were extremely high compared to the other two cell lines (OUR-10 and NC65), which might be one of the reasons why DEX could not inhibit the growth of cells to only a limited extent in Caki-1 cells. However, the growth inhibition of Caki-1 cells was demonstrated by antisense nucleotide against IL-6 gene, which suggests that IL-6 is also an important growth-promoting cytokine in Caki-1 cells (17).

In our series of experiments, we demonstrated, for the first time, the *in-vivo* growth inhibition of RCC cells by DEX using nude mice. The growth-inhibitory effect of DEX was more remarkable *in vivo* than *in vitro*. Immunohistochemical study revealed the translocation of the GR from the cytosol to the nucleus, as in the cell-culture experiments, suggesting that DEX acts through transcriptional activation of GR. As mentioned above, VEGF and IL-8 are also target genes for NF-κB. Their concentrations in conditioned medium and cell lysates decreased following DEX treatment in a dose-dependent manner, except for IL-8 in Caki-1 cells. The reason why IL-8 production in Caki-1 cells did not respond to DEX is not known. Lower expression of GR may be one of the reasons. VEGF is a well-known angiogenic factor important for neovascularization when a tumor grows *in vivo*. Decreased VEGF might be also one of the most important factors for growth-inhibition of RCC caused by DEX treatment (19). This could be why RCC growth suppression was more remarkable *in vivo* than *in vitro*.

In conclusion, DEX is a possible therapeutic agent for advanced RCC via suppression of IL-6, IL-8 and VEGF production through inhibition of nuclear translocation and subsequent inactivation of NF-κB. However, as growth-inhibitory effects of DEX are less marked than newly developed kinase inhibitors, we think DEX can be best used in combination with other drugs.

REFERENCES

1. Motzer, R.J.; Russo, P. Systemic therapy for renal cell carcinoma. J. Urol. 2000, 163, 408–417.

2. Pizzocaro, G..; Piva, L.; Colavita, M.; Ferri, S.; Artusi, R.; Boracchi, P.; Parmian, G.; Marubini, E. Interferon adjuvant to radical nephrectomy in Robson stages II and III renal cell carcinoma: a multicentric randomized study. J. Clin. Oncol. 2001, 19, 425–431.

3. Clark, J.I.; Atkins, M.B.; Urba, W.J.; Creech, S.; Figlin, R.A.: Dutcher, J.P.; Flaherty, L.; Sosman, J.A.; Logan, T.F.; White, R.; Weis, G.R.; Redman, B.G.; Tretter, C.P.; McDermott, D.; Smith, J.W.; Gordon, M.S.; Margolin, K.A. Adjuvant high-dose bolus interleukin-2 for patients with high-risk renal cell carcinoma: a cytokine working group randomized trial. J. Clin. Oncol. 2003, 21, 3133–3140.

4. Yagoda, A.; Abi-Rached, B.; Petrylak, D. Chemotherapy for advanced renal-cell carcinoma: 1983-1993. Semin. Oncol. 1995, 22, 42–60.

5. Mizutani, Y.; Bonavida, B.; Koishihara, Y.; Akamatsu, K.; Ohsugi, Y.; Yoshida, O. Sensitization of human renal cell carcinoma cells to cis-diamminedichloroplatinum(II) by anti-interleukin 6 monoclonal antibody or anti-interleukin 6 receptor monoclonal antibody. Cancer Res. 1995, 55, 590–596.

6. Motzer, R.J.; Michaelson, M.D.; Redman, B.G.; Hudes, G.R.; Wilding, G..;Figlin, R.A.; Ginsberg, M.S.; Kim, S.T.; Baum, C.M.; DePrimo, S.E.; Li, J.Z.; Bello, C.L.; Theuer, C.P.; George, D.J.; Rini, B.I. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. J. Clin. Oncol. 2006, 24, 16–24.

7. Miki, S.; Iwano, M.; Miki, Y.; Yamamoto, M.; Tang, B.; Yokokawa ,K.; Sonoda, T.; Hirano, T.; Kishimoto, T. Interleukin-6 (IL-6) functions as an in vitro autocrine growth factor in renal cell carcinomas. FEBS Lett. 1989, 250, 607–610.

8. Blay, J.Y.; Negrier, S.; Combaret, V.; Attali, S.; Goillot, E.; Merrouche, Y.; Mercatello, A.; Ravault, A.; Tourani, J.M.; Moskovtchenko, J.F.; Phillip, T.; Favrot, M. Serum level of interleukin 6 as a prognosis factor in metastatic renal cell carcinoma. Cancer Res. 1992, 52, 3317–3322.

9. De Bosscher, K.; Vanden, Berghe, W.; Vermeulen, L.; Plaisance, S.; Boone, E.; Haegeman, G. Glucocorticoids repress NF-kappaB-driven genes by disturbing the interaction of p65 with the basal transcription machinery, irrespective of coactivator levels in the cell. Proc. Natl. Sci. USA 2000, 97, 3919–3924.

10. Oya, M.; Takayanagi, A.; Horiguchi, A.; Mizuno, R.; Ohtsubo, M.; Marumo, K.; Shimizu, N.; Murai, M. Increased nuclear factor-kappa B activation is related to the tumor development of renal cell carcinoma. Carcinogenesis 2003, 24, 377–384.

11. Matsuda, M.; Osafune, M.; Nakano, E.; Kotake, T.; Sonoda, T.; Watanabe, S.; Hada, T.; Okochi, T.; Higashino, K.; Yamamura, Y.; Abe, T. Characterization of an established cell line from human renal carcinoma. Cancer Res. 1979, 39, 4694–4699.

12. Nakano, E.; Tada, Y.; Fujioka, H.; Matsuda, M.; Osafune, M.; Kotake, T.; Sato, B.; Takaha, M.; Sonoda, T. Hormone receptor in renal cell carcinoma and correlation with clinical response to endocrine therapy. J. Urol. 1984, 132, 240–245.

13. Coleman, R.E. Glucocorticoids in cancer therapy. Biotherapy 1992, 4, 37–44.

14. Nishimura, K.; Nonomura, N.; Satoh, E.; Harada, Y.; Nakayama, M.; Tokizane, T.; Fukui, T.; Ono, Y.; Inoue, H.; Shin, M.; Tsujimoto, Y.; Takayama, H.; Aozasa, K.; Okuyama, A. Potential mechanism for the effects of dexamethasone on growth of androgen-independent prostate cancer. J. Natl. Cancer Inst. 2001, 93, 1739–1746.

15. Nishimura, K.; Nonomura, N.; Yasunaga, Y.; Takaha, N.; Inoue, H.; Sugao, H.; Yamaguchi, S.; Ukimura, O.; Miki, T.; Okuyama, A. Low doses of oral dexamethasone for hormone-refractory prostate carcinoma. Cancer 2000, 89, 2570–2576.

16. Takenawa, J.; Kaneko, Y.; Okumura, K.; Yoshida, O.; Nakayama, H.; Fujita, J. Inhibitory effect of dexamethasone and progesterone in vitro on proliferation of human renal cell carcinomas and effects on expression of interleukin-6 or interleukin-6 receptor. J. Urol. 1995, 153, 858–862.

17. Alberti, L.; Thomachot, M.C.; Bachelot, T.; Menetrier-Caux, C.; Puisieux, I.; Blay, J.Y. IL-6 as an intracrine growth factor for renal carcinoma cell lines. Int. J. Cancer 2004, 111, 653–661.

18. Blay, J.Y.; Rossi, J.F.; Wijdenes, J.; Menetrier-Caux, C.; Schemann, S.; Negrier, S.; Philip, T.; Favrot, M. Role of interleukin-6 in the paraneoplastic inflammatory syndrome associated with renal-cell carcinoma. Int. J. Cancer 1997, 72, 424–430.

19. Iwai, A.; Fujii, Y.; Kawakami, S.; Takazawa, R.; Kageyama, Y.; Yoshida, M.A.; Kihara, K. Down-regulation of vascular endothelial growth factor in renal cell carcinoma cells by glucocorticoids. Mol. Cell Endocrinol. 2004, 226, 11–17.

6. 子宮頸がん再発

— pelvic exenteration —

笠松高弘*・恩田貴志*・澤田守男*・加藤友康*
国立がんセンター中央病院婦人科*

Key Words/子宮頸がん局所再発・pelvic exenteration

要旨

子宮頸がんの局所再発で救命可能な例は希少であり以下の2つの場合である．中央再発で，pelvic exenteration が可能な症例と，未照射野内の表層性または径の小さな腫瘍で根治照射が可能な症例である．これらの場合以外は，抗癌剤治療を含め，すべての治療が palliation 目的の治療となる．pelvic exenteration は障害の大きな治療法であり，その選択には充分に慎重な検討が必要である．しかしながら照射野内再発に対する唯一の根治可能な治療手段であり，婦人科がん治療医ならば，慎重になりすぎ適応のある患者に生存の機会を失わせてしまうことは決してしてはならない．

はじめに

他の癌種の多くがそうであるように，子宮頸がん（頸がん）もまた再発すると，集学的治療を行ってもほとんどの場合患者は早期に死亡し，根治や5年以上の長期生存ができる例は癌専門施設でも数%程度のみである．すなわち患者にとって再発は近いうちの死を意味することは言うまでもない．また再発の治療には初回治療ほどの標準治療というものはないし，ましてランダム化比較試験ほどのエビデンスのある治療法もない．しかし，そこには一定の法則のような

ものは存在し，選択すべきある程度定型的な治療は存在する．再発治療だからといって何でもやってみるという適当な治療は許されるものではない．

本稿では頸がんの局所（骨盤内）再発に対する治療成績の現状，特に pelvic exenteration（骨盤内臓器全摘術）の位置づけ，適応などについて当院（国立がんセンター中央病院）の治療成績[1][2] を紹介しながら概説を加える．なお，pelvic exenteration そのものの手技の詳細は他の骨盤外科の成書を参考にされたい．

表1 患者背景 （n = 664）   文献1）より引用

Mean age	52 years （range, 22-86）		
Histologic subtypes	Squamous	493 （74%）	
	Adeno	117 （18%）	
	Adenosquamous	38 （6%）	
	Others	16 （2%）	
FIGO stage	I B	353 （53%）	
	II A	65 （10%）	
	II B	108 （16%）	
	III A	3 （0.5%）	
	III B	119 （18%）	
	IVA	16 （2.5%）	
Treatment modalities	Surgery	471 （71%）	
		Radical hysterectomy	419
		Simple Histerctomy	46
		Pelvic exenteration	6
		Postoperative adjvant radiotherapy	156
	Radiotherapy	193 （29%）	


```
再発 （一） 471/664 （71%） ← n＝664
                              ↓
                 再発 （＋） 193/664 （29%）
        ↙               ↓                    ↘
  骨盤内            骨盤外                骨盤内・外
  67/193 （35%）    109/193 （56%）       13/193 （7%）

・原病死 52/67 （76%）   ・原病死 96/109 （88%）   ・原病死 13/13 （100%）
・他因死 4/67 （6%）      ・他因死 2/109 （2%）
・生存 有病 3/67 （4%）   ・生存 有病 3/109 （3%）
      無病 8/67 （12%）         無病 7/109 （6%）

                ＊再発部位不明 4，死因不明 1
```
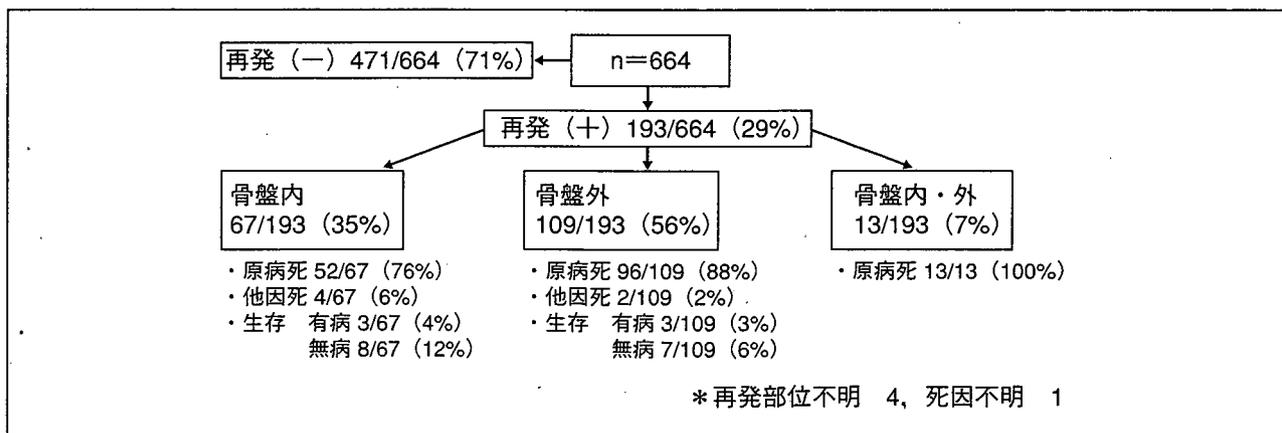
図1 頸がん I B-IVA 期患者の予後の Overview   文献2）より引用

国立がんセンター中央病院における頸がん局所再発例の overview

1. 対象

1989 ～ 2000 年に当科で治療した I B-IVA 期 664 例を後方視的に検討したものである．初回治療の治療原則は，I B － II B （III A） 期は広汎子宮全摘術または放射線治療 （外照射＋高線量率腔内照射），III B （III A）－ IVA 期は放射線治療である．また術後照射の基準はリンパ節転移陽性または傍子宮結合織浸潤陽性の2点である．

2. 結果

患者背景は，表1のごとくである．約70%に手術，30%に放射線治療が行われ，術後照射の率は37%であった．なお観察期間は平均68 （1 ～ 166）カ月であった．

これら664例の5年累積生存率は，I B 期 84%，II A 期 78%，II B 期 65%，III A 期 67%，IVA 期 38% であった．予後の overview を図1に示す．664例のうち193例 （29%） が再発し，うち35%が骨盤内単独再発であった．再発治療後の無病生存例は骨盤内再発で12% （8/67例），

表2　骨盤内再発患者背景　（n＝67）　　　　　　　　　　　　　　　　　　　　　　　　　　文献1）より引用

Location of tumor	n (%)	Prior radiotherapy	n	Treatment modality for recurrence	n	Status[a]	n (mo)[b]
Central pelvis	24 (35%)	Outside the irradiated field[e]	4	Radiotherapy	4	NED[c]	1 (71)
		Inside the irradiated field[f]	9	Radiotherapy	3	NED	1 (62)
				Pelvic exenteration	2	NED	1 (142)
				Palliative surgery	2		
				Not done	2		
		Not done[g]	11	Radiotjerapy	8	NED	3 (70, 78, 86)
				Pelvic exenteration	3	NED	2 (61, 24)
Pelvic wall involvement	43 (65%)	Inside the irradiated field	25	Radiotherapy	5		
				Pelvic exenteration	1		
				Palliative surgery	1	AWD[d]	1 (34)
				Chemotherapy	6	AWD	2 (17, 23)
				Not done	12		
		Not done	18	Radiotherapy	9		
				Pelvic exenteration	2		
				Chemotherapy	4		
				Not done	3		

[a] Blank represents dead of disease. [b] Survival after initial treatment. [c] No evidence of disease [d] Alive with disease.
[e] The recurrent tumor was located outside the field of irradiation that had been performed for the initial treatment
[f] The recurrent tumor was located inside the field of irradiation that had been performed for the initial treatment
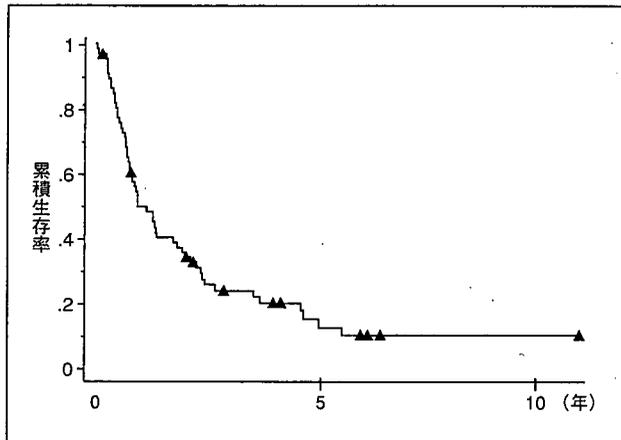[g] Patients who did not receive radiotherapy for treatment of initial therapy

図2　骨盤内再発後の予後　（n＝67）

骨盤外再発で6%（7/109例）であった．いずれも5年以上の長期生存である．他は少数の終末期緩和ケア中の患者を除き全例死亡していた．

　この骨盤内再発67例の内訳は，ⅠB期26例，ⅡA期6例，ⅡB期13例，ⅢA期1例，ⅢB期18例，ⅣA期3例であり，初回治療として63%（42例）に手術，うち12%（12例）に術後照射が行われており，残り37%（25例）は放射線治療例であった．組織型は扁平上皮がん67%，腺がん21%，腺扁平上皮がん6%，その他特殊型であった．

　これら67例の臨床像の詳細を再発部位，および照射野内再発か未照射野からの再発かの観点から示したものが**表2**である．再発部位としては35%が中央再発（central recurrence），65%は再発腫瘍が骨盤壁から再発したか，または骨盤壁を浸潤したいわゆる側方再発（pelvic side wall recurrence）であった．この側方再発例には救命できた例は存在しなかった．救命可能であった例の再発部位はいずれも中央再発であった．これらの生存者は皆，再発後3年以上の長期無病生存例である．67例全体の再発後の5年累積生存率は15%であり，50%生存期間は1年であった（**図2**）．

　この救命可能であった8例の臨床像を**表3**に示した．3例（症例1, 7, 8）は pelvic exenteration が可能であった例である．4例は外照射と小線源治療の併用により充分な根治的放射線治療が可能であった例である．この4例のうち3例には照射歴はなく（症例4, 5, 6），1例は放射線治療例であるが未照射野からの再発であった（症例2）．なおこの4例の再発腫瘍は，2 cm以下の小型の腫瘍か，または腟壁表面に浅く広がった腫瘍であった．なお症例3は術後照射野内の腟断端再発例で pelvic exenteration の適応症例であったが，患者の拒否により，過線量を承知で腔内照射を追加した例である．幸いにも

表3. 生存者患者背景　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　文献1) より引用

Patient no.	FIGO Stage	Histological subtype	Primary therapy	Recurrent Site	Recurrence free interval (mo)	Salvage therapy	Survival after recurrence (mo)
1	ⅡA	Squamous	Radiotherapy	Cervix, Bladder	7	Anterior exenteration	135
2	ⅢB	Squamous	Radiotherapy	Vaginal wall	21	Radiotherapy	50
3	ⅠB	Adenosquamous	Radical hysterectomy + radiotherapy	Vaginal stump	12	Radiotherapy	50
4	ⅠB	Squamous	Radical hysterectomy	Vaginal stump	7	Radiotherapy	79
5	ⅠB	Squamous	Radical hysterectomy	Vaginal wall	2	Radiotherapy	76
6	ⅠB	Adenosquamous	Radical hysterectomy	Vaginal wall	18	Radiotherapy	52
7	ⅠB	Adenocarcinoma	Radical hysterectomy	Rectum, Bladder	26	Total exenteration	35
8	Ⅲb	Glassy cell	Anterior exenteration + Chemotherapy	Rectum	44	Posterior exenteration	80

再発後 50 カ月無病生存しているが，高度の晩期放射線性膀胱障害により膀胱カテーテル留置を余儀なくされている.

図3　救命可能な対象症例

どのような場合が救命の可能性をもっているか

前項の当科の症例シリーズを見ると，局所再発した場合，ⅠB－ⅡB期の 13%，ⅢB 期の11%しか救命できていないことがわかる．当院のセカンドオピニオン外来には多くの救命不可能な再発患者さんが相談に来られるが，時に延命効果はもちろん，抗癌剤の有害事象が前面に出て症状緩和効果すら得られていないと思われる抗癌剤治療を，first line, second line, third line と延々と続けている例を散見する．また逆に pelvic exenteration や放射線治療のよい適応で救命の可能性があるのにその検討がされていないこともある.

もちろん再発治療には標準治療と言えるほどのエビデンスをもった治療体系はないが，先達の貴重な後方視的調査研究等を鑑みれば，それでもある程度の法則性や，第一に選択すべき治療法というものは存在する．救命の可能性がある場合には患者からその機会を奪っては絶対にならないし，また根治が得られない場合は，手術，抗癌剤，放射線といった抗癌治療を含め，すべての治療は palliation 目的の治療と位置づ
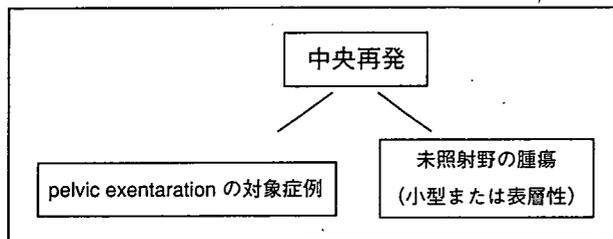
けて，命の長さに限りがあるにせよ，そのなかでよりよい状態で一日でも延命ができるようサポートしなくてはならない．再発の治療にあたる場合にも，選択した治療が根治目的なのか palliation 目的なのかを患者も治療医もはっきり認識して治療に望むことが必要である.

それではきわめて予後の悪い頸がんの骨盤内再発において，いかなる場合に救命の可能性があるのか．前項の当院の症例シリーズや既存の調査研究，review 等を踏まえると図3の二つの場合であると考える．まず，中央再発であることが前提である．側方再発の救命はいずれの治療法をとっても困難であるとされている．その理由として漠然と，側方再発自体が metastatic systemic disease で中央再発とは生物学的に異なった性質・態度をとるものである，と説明されてきた．1990 年代に，比較的小さな側方再発に対し，手術により腫瘍体積を可及的に縮小せしめた後，術中にアプリケーターを残存部に留置し，高線量率照射を追加する High-Dose-Rate Intraoperative Radiation Therapy （HDR-IORT）で中央再発例に近い長期の局所制御も可能であ

るとする試験治療の報告もみられたが，その後普及はしていない[3][4]．なお，プラチナ製剤を中心とする抗癌剤治療は，現在の奏功率ではすべての場合において palliation 目的の手段と位置づけられる．

1）根治的放射線治療が可能ということは，術後照射などの照射歴がないか，あっても照射野外の再発であるということになるが，それにもさらに条件がつく．Ito ら[5]の腟断端部再発に対する高線量率腔内照射の腫瘍サイズ別の治療成績を見ると，その10年生存率は，腫瘤として触れない場合が72%，3cm以下で48%，それ以上では0%となっている．ということは前述の当院の症例シリーズでも示されるように，実際には腟壁から外方発育的に発生する小型の腫瘤か，面積は比較的広くとも腟壁表層を這うように存在し腫瘤を形成していない再発形式ということになる．このように都合よく再発することはきわめて少ないが，逆にいえば，この条件を満たす再発と診断した場合，適切な放射線治療をせずにおくことは許されないと考えねばならない．もちろん，実際の治療には放射線治療専門医による組織内照射，腔内照射などの小線源治療や3次元原体照射などを用いた特殊な治療が必要で，ただ漫然と外照射を施行すればよいということではないので，自分の施設にそれがないときは，これらが可能な施設へ治療を依頼することが必要なのは言うまでもないことである．

2）次の pelvic exenteration は，照射野内再発に対する唯一の救命の可能性のある方法である．

表4　pelvic exenteration の患者選択基準

・中央再発	除外：	水腎症
		下肢浮腫
		坐骨神経痛
・再発期間	2年以上	
・リンパ節転移	なし	
・組織型	除外：	腺がん？

日本の場合，広汎子宮全摘術後に術後照射を行っていることが多いので，実際には広汎子宮全摘後の直腸，膀胱，腟で囲まれる範囲の再発がその適応となることが多い．

pelvic exenteration の患者選択基準

本術式は尿路変更，人工肛門造設を伴う大きな手術である．加えて頸がんでは，すでに広汎子宮全摘術が行われ，さらに全骨盤照射，腟断端腔内照射が行われた患者がその対象となることが多いため，術野の中心となる骨盤中央部の再発腫瘍周囲は高度に繊維化している．また，腸管，尿管も放射線の照射野に入っているため，その吻合等には，高度な技術と経験が必要で，これらに長けた泌尿器科医，大腸外科医などの骨盤外科医チームがいなければ安全かつ根治性の高い手術ができるものではない．術後障害を残したり，根治性が損なわれたりすることがあってはならない．不成功に終わると，多大な身体的障害のみならず，精神的苦痛を大きく残す．したがって患者選択にはきわめて慎重な配慮が必要である．

一般的にいわれている選択基準は表4のごとくである．患者選択の絶対条件は中央部再発であるが，その評価に迷うことは多い．放射線治療による病巣周囲組織の繊維化は，内診時に骨盤壁への浸潤との鑑別を要することがある．たとえば5cmの腫瘤であっても切除してみるとがんの部分は中心部の2～3cm程度で，周囲は繊維組織であることも多い．水腎症，坐骨神経痛および下肢浮腫はいずれもすべてではないが，側方浸潤を示唆する所見として重要である．自験例でも不成功例にこれらの症状を高率に認めている[2]．

また，術前だけでなく開腹時にも腹膜細胞診，

表5 pelvic exenteration 症例 （1973～2000, n＝17） 文献2）より引用

No.	FIGO	組織型	初回治療	再発時期 mo.	術式	切除度	予後 mo. （PE 後）	
1	Ⅱa	扁平	放治	6	APE	完全	NED	142 （135）
2	pT3bN1	すりガラス	APE	44	PPE	完全	NED	124 （80）
3	pT1bN0	腺	ARH	26	TPE	完全	NED	61 （34）
4	Ⅱa	扁平	放治	15	TPE	完全	敗血症	23 （6）
5	Ⅲb	扁平	放治	9	TPE	完全	脳出血	25 （11）
6	ⅠB	扁平	放治	10	TPE	完全	肺梗塞	71 （49）
7	Ⅱa	扁平	放治	3	TPE	不完全	遠隔	12 （2）
8	pT1bN0	腺	ARH	17	TPE	完全	遠隔	21 （3）
9	pT2bN1	腺	ARH	14	TPE	完全?	遠隔	18 （4）
10	pT1bN0	腺	ARH	10	TPE	完全	遠隔	15 （4）
11	pT1bN0	扁平	ARH	11	TPE	不完全	局所	15 （6）
12	pT1bN1	扁平	ARH, WP	13	TPE	不完全	局所	60 （6）
13	pT1bN0	腺	ARH	38	TPE	不完全	遠隔	52 （10）
14	pT3bN1	腺	TAH, WP	6	TPE	完全	局所	33 （16）
15	pT2bN1	扁平	ARH, WP	5	TPE	完全	遠隔	27 （21）
16	pT2aN0	腺	ARH, WP	12	TPE	完全	遠隔	48 （33）
17	pT1bN0	腺	ARH	23	TPE	完全?	遠隔	121 （94）

APE:anterior pelvic exenteration, PPE:posterior pelvic exenteration,
TPE:total pelvic exenteration, WP:whole pelvis radiation,

腫大リンパ節の生検を行い評価することが必要である．これらが陽性の場合，原則手術は中止すべきである．

再発までの期間としては，2年以上の例の予後が良好とされている．

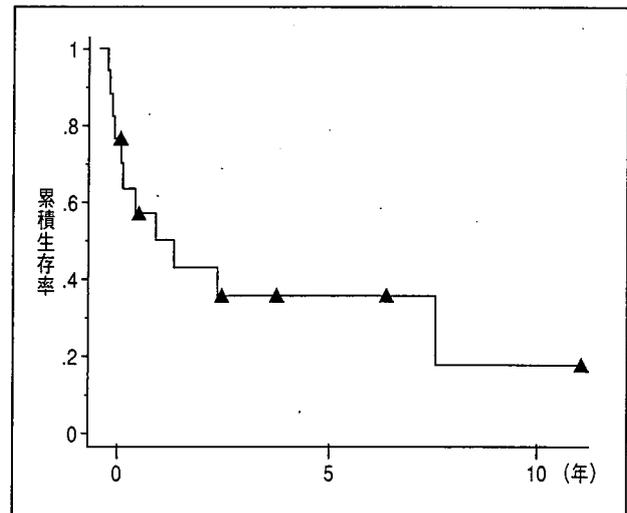組織型では，腺がんには遠隔再再発が多く好ましくないとする意見も多いが，治療成績が扁平上皮がんと同等する報告[6]もあり，結論付けられない．当院の救命例には，少なからぬ非扁平上皮がんが含まれている[2]．

図4 pelvic excenteraion 後の予後 （n＝17）

pelvic exenteration の治療成績

主要施設での頸がん局所再発に対する pelvic exeneration 後の5年生存率はおよそ 20～60%であり，手術関連死は 10%以下である[7]~[10]．この生存率の数字は，乳がんのような長期に担癌生存する癌種を除き，再発治療としては満足すべき治療手段であるといえる．

当院の症例シリーズを表5に，再発後の生存曲線を図4に呈示した．exenteration 後の5年生存率は 36%，50%生存期間は 16 カ月，治療関連死は 5.8%であった．生存例はいずれも無病生存で，exenteration 後 3～10 年経過している．

表6 切除度と術前所見 n (%)　文献2) より引用

	完全切除 11	不完全 切除 4
水腎症	2/11 (18)	4/4 (100)
坐骨神経痛	2/11 (18)	4/4 (100)

治療関連死は1例であるが，術後骨盤内膿瘍で敗血症に至った症例である．内科疾患による他因死は2例あるが，再発は認めなかった．画像診断や術後管理，よい palliation 手段が今ほどではない古い時代の症例も多く含まれており，現在の目からすればすべてが前項の症例選択基準を満たしているとはいいがたいが，生存例については，その他の方法では救命困難であったと思われる．

　術後病理標本による完全切除割合は76%であった．不完全切除4例にはいずれも顕微鏡的な側方浸潤があった．切除度と術前所見の関係の一つとして，水腎症と坐骨神経痛についてみると，不完全切除例には全例これらの症状を認めたが完全切除例では18%であった（表6）．やはり教科書的な症例選択基準は重視すべきであり，その適応にはきわめて慎重な検討が必要である．

palliation としての pelvic excenteration

　今まで述べてきたように，pelvic excenteration は根治の可能性がある場合にのみ適応となる治療法である．一方，palliation を目的とした pelvic excenteration もあり得るとする議論は散見される．すなわち，頸がんが局所再発すると，いずれ膀胱腟瘻，直腸腟瘻が生じ，そこに感染が起こり悪臭のある分泌物が常時排泄されるようになる．やがて性器出血がおこり患者は大量性器出血死の恐怖にさらされる．このような悲惨な状態になるのを避ける目的で，根治の可能性がない場合でも，早期に pelvic exenteration を行うことによって局所の状態をよりよく保とうという考え方である．簡単なストーマ造設程度の処置では，このような状態は思ったようには改善しないからである．1976年に Deckers PJ ら[11] が，進行・再発頸がんなどに局所状態の改善をめざし，palliation としての pelvic exenteration を行い，患者とその家族のいわゆる "quality of life" の改善を図れたとする18例の症例シリーズを発表したのが端緒だと思われる．その論文の冒頭には次のように書かれている，"The rigid demand that cure be the only end of pelvic exenteration fails to recognize that many serious oncologic efforts are, at best, palliative and designed toward improvement in the quality of life for a defined, albeit brief, interval of time. Exenteration of the pelvic viscera can be extremely useful in this regard and if the published morbidity and mortality rates can be reduced, then we believe that use of this procedure as palliation should be advocated."．この論文は単なる症例報告でなく，誠実な腫瘍外科医の深い省察に基づく見解と理解されるが，やはり palliation 目的の pelvic exenteration は慎むべきである．palliation 目的としてはあまりに侵襲とリスクが大きすぎるという至極当然な理由はさておき，医師の行う "symptom control" と患者側からみた "quality of life" を混同している恐れがあるからである．医療者側からみれば確かに悲惨な局所の状態を回避でき（symptom control），よかれと思って行ったことが，必ずしも終末期になって患者側からみてこの処置を受けて本当によかったと感じること（quality of life）と，必ずしも等価ではないからである．