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The pinhole SPECT for animal model of bone metastasis with SPC-A-1BM human pulmonary adenocarcinoma bone metastasis cell line

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Abstract The study was to investigate the role of pinhole single photon emission computed tomography (SPECT), the human pulmonary adenocarcinoma bone-seeking metastasis cell line SPC-A-1BM was used. These cells form typical osteolytic bone metastases when inoculated into the arterial circulation of NIH-Beige-Nude-XD (BNX) mice *via* the left ventricle. In order to evaluate the irradiation impact of ^{99m}Tc-MDP versus X-ray on cells growth, we used six groups of SPC-A-1BM cells in our imaging scheme and irradiated by various doses of ^{99m}Tc-MDP (37, 74, 111, 370, 740 MBq) and X-ray(40 kV, 2 mA, 6 s) respectively. The cell's number of each group was well recorded in different exposure time（4, 8, 12, 24, 48, 72, 96 hours）. After that, SPC-A-1BM cells (1×106) were inoculated into the mice *via* left ventricle. We compared the results obtained with those different doses of ^{99m}Tc-MDP using pinhole SPECT and conventional X-ray skeletal surveys. The data show that the cell-survival number of 111 MBq group has insignificant difference with that of X-ray and the dose is adequate to have an ideal image. Besides, it is important that the chromosome of the cells in the group of 111 MBq showed no irradiation-related damages in our test. These results implied that ^{99m}Tc-MDP pinhole SPECT may provide another way other than conventional X-ray skeletal surveys in detecting bone metastasis of pulmonary adenocarcinoma in BNX mice.

Key words ^{99m}Tc-MDP, Pinhole SPECT, Radiation, Pulmonary adenocarcinoma, Bone metastasis, Animal model

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1 Introduction

In detecting bone metastasis, at least a 5%–10% change in the ratio of lesion to normal bone is required to detect an abnormality on bone scintigraphy (BS)[1,2]. It has been estimated that BS can detect malignant bone lesions 2~18 months earlier than plain radiography (XR)[1], and BS sensitivities in detecting bone metastases vary between 62% and 100% with a specificity of 78%~100%[3]. This is because the alteration in osteoblastic action and/or blood supply occurs at an earlier stage than the difference in bone density needed to produce a radiographic change. Technetium-99m methylene dihosphonate (^{99m}Tc-MDP) is the most widely used bone scan agent in BS, giving optimal contrast between normal and diseased bone[4]. The use of pinhole collimator, instead of parallel hole collimator, can improve the sensitivity of planar

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scintigraphy[5]. While, only few studies evaluated P-SPECT in hyperparathyroidism (HPT)[6-8].

Lung cancer is a common cause of death in the world. Approximately 75% of human lung cancers are diagnosed as non-small-cell-lung-cancer (NSCLC)[9]. Pulmonary adenocarcinoma, the most common form of NSCLC[10], is of high incidence, high bone metastasis potential and poor survival. It is an urgent task to identify the bone metastasis as early as possible, so as to determine the full extent of disease, to evaluate the presence of complications that may accompany malignant bone involvement (including pathologic fractures and spinal cord compression), to monitor response to therapy and to guide biopsy if histological confirmation is needed[1,3,9,11].

Unfortunately, to date, few data have been reported on the use of this scintigraphy studies for evaluating the extent of bone metastasis in animal models, whereas it has been reported that bone lesions were detected by X-ray analysis in breast cancer bone metastasis[12,13]. But the 10~12 weeks after the injection of tumor cells is usually beyond the survival time of animals and the rate of sensitivity is relatively low[13]. As BS is advantageous over X-ray radiography in detecting bone metastasis, it is necessary to develop a predictive test with sufficient sensitivity to allow suitable dose of the scintigraphy agent to be used for each case. Studies involving ionizing radiations, as is the case in nuclear medicine, require special consideration to comply with the ALARA (as low as reasonably achievable) principle[14]. Also, biological irradiation effects on normal tissues, such as gene mutation, chromosomal rearrangement, cellular transformation, cell death, etc, will have to be avoided[15].

The aim of this study was to investigate the role of ^{99m}Tc-MDP pinhole SPECT for detecting bone metastasis of human pulmonary adenocarcinoma in BNX mice. The results obtained are compared with BS and conventional X-ray skeletal surveys.

2 Materials and methods

2.1 Cell culture

The SPC-A-1BM human pulmonary adenocarcinoma bone metastasis cell line was used as a model system, which was established in our laboratory[16] (Patent No：ZL200410093062.8). The cells were routinely cultured in RPMI-1640 medium (Invitrogen Corp., USA) supplemented with 10% fetal bovine serum (Gibco Corp., USA) at 37℃ in a humidified atmosphere with 5% CO_2. The cells were washed for several times, and placed in sterile phosphate-buffer solution shortly before implantation.

2.2 Animals

In this study, the BNX mice[17] (Shanghai Cancer Institute, China) were used. All animals were maintained under pathogen-free conditions and handled according to China regulations for animal experimentation. All manipulations were conducted under aseptic conditions using a laminar flow hood.

2.3 Irradiation procedure

The SPC-A-1BM cells were inoculated at densities of 3.3×10^5 into 42 culture flask (Corning Corp., USA) divided into two groups. The control group were randomly divided into two subgroups (seven culture flasks each), with the negative control subgroup receiving no specific treatment (non-radiation treated) and the positive control subgroup being exposed to 40 kV 2 mA X-ray for 6 s[18]. The treated groups, with the remaining 28 culture flasks, were given ^{99m}Tc-MDP of 37 MBq, 111 MBq, 370 MBq and 740 MBq (seven culture flasks each). The cells were evaluated for different exposure hours (4, 8, 12, 24, 48, 72 and 96 h) and the numbers were well counted under an inverted microscope (Olympus, Japan). The counting was repeated for five times and expressed in mean ± SD.

2.4 Chromosome analysis on SPC-A-1BM cell

The groups of SPC-A-1BM cells in the stage of proliferation were selected, stirred and spread, and treated with 10 μg/mL colchicines for 4~6 h. Cell suspension was collected, and Giemsa stained after hypotonic treatment. The cells with well-dispersed chromosomes in metaphase were selected. The chromosome number was counted, and morphology of chromosomes was observed.

2.5 Experimental bone metastasis model

BNX mice of 8 to 10-week-old were anesthetized with intra-peritoneal injection of 37.5 mg·kg^{-1} pentothal sodium and intra-muscle injection of 75 mg·kg^{-1} ketamine hydrochloride. For inoculation, cells were recovered from the cell culture flask using phosphate-buffered solution (PBS). We inoculated 1×10^6 SPC-A-1BM cells suspended in 100 μL of sterile PBS through the inter-costal space into the left ventricle of the mice. On day 14 after the cell inoculation, the animals were injected intravenously through the tails with 37 MBq, 111 MBq, 370 MBq or 740 MB of ^{99m}Tc-MDP (GMS, Co., Ltd, Shanghai, China) suspended in 100 μL of sterile PBS. About 5 h later, assessment of bone metastasis was monitored with pinhole SPECT (GE, Millennium VG, USA, *d*=1mm), which was operated at matrix size of 256×256, 2.67 zooming to 3×10^5 counts.. Each of the inoculations used a separate 29-gauge needle (Terumo, Japan). The bone metastasis sites detected in BS were segregated and stained with hematoxylin and eosin (H&E) for pathological studies.

2.6 Statistical analysis

The difference in radiation response between groups was evaluated using the One-Way ANOVA with SPSS 11.0 software. Statistical significance was defined as $p<0.05$.

3 Results

3.1 The cells survival number

The results are summarized in Table 1. In the negative control group, cells number increased with time, and the cells survival number doubled in 24 h comparing with the initial. Although the cell has high ability of proliferation, the number decreased gradually with increasing dose of ^{99m}Tc-MDP in the same period of time.

Table 1 The impact of ^{99m}Tc-MDP on cell number ($\times10^4$, in mean ±SD)

Time / h	The negative control group (no treatment at all)	The ^{99m}Tc -MDP group			
		37 MBq	111 MBq	370 MBq	740 MBq
0	33.0	33.0	33.0	33.0	33.0
4	40.4±4.2	38.6±5.0	30.6±5.6	22.9±3.6	18.0±3.2
8	42.8±3.7	41.0±6.6	34.0±4.3	22.6±1.7	16.7±2.9
12	53.0±3.9	50.0±5.3	47.0±4.8	30.0±2.3	22.2±2.3
24	80.0±6.0	77.6±8.4	61.7±6.6	54.4±3.6	24.8±5.6
48	121.0±24.7	115.0±10.8	104.7±13.1	70.6±20.3	30.0±5.3
72	150.0±18.9	142.6±23.8	128.8±13.3	75.6±7.7	34.0±4.9
96	166.6±8.9	149.0±26.8	144.5±15.4	81.0±14.0	38.9±6.1

3.2 ^{99m}Tc-MDP versus X-ray on the cells survival number

The survival number of groups irradiated by 740 MBq and 370 MBq ^{99m}Tc-MDP were respectively fewer than those of the negative control group, the 37 MBq group, the 111 MBq group and the X-ray group ($p<0.05$), whereas the cells survival number in the 111 MBq group was insignificantly different from the X-ray group ($p=0.780$) and the 37 MBq group ($p=0.693$), indicating that the cell survival number were similar after they were irradiated with ^{99m}Tc-MDP of 111 MBq and 37 MBq or 40 kV 2 mA X-rays for 6 s (Fig.1).

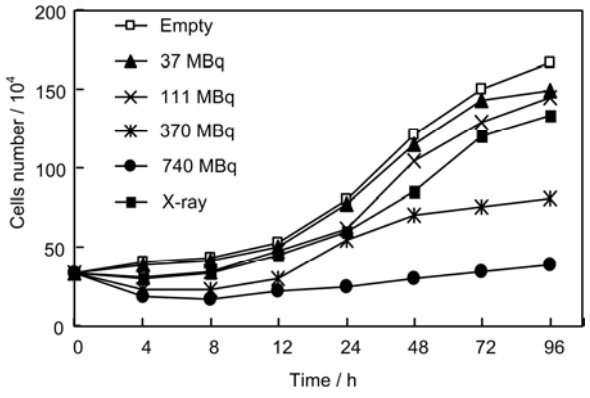

Fig.1 Cells survival number after various doses of ^{99m}Tc-MDP or 40 kV 2 mA X-rays for 6 s at room temperature.

3.3 Chromosome analysis

Two kinds of chromosome morphology could be observed under oil immersion lens. The first type was condensed and could be counted in total when the band could not be seen (Fig.2). The second type was chromosome that spread completely in the center of equatorial plate and was in metaphase in which total numbers and bands were seen relatively clearly (Fig.3). There were 6 cells having diploid chromosome, 84 cells having chromosome between diploid and triploid, 10 cells having multiploid chromosome in all 100 cells. It was observed that the number of chromosome 5, 7, 10, 13, 17, 21 and 22 increased, whereas the number of chromosome 8, 16 and 18 decreased. Besides, the ninth chromosome was deletion. However, in our study the karyotype of the SPC-A-1BM cells in the 111 MBq group was the same as that of homo-sapiens, which showed granule or rod-shape (Figs.2 and 3).

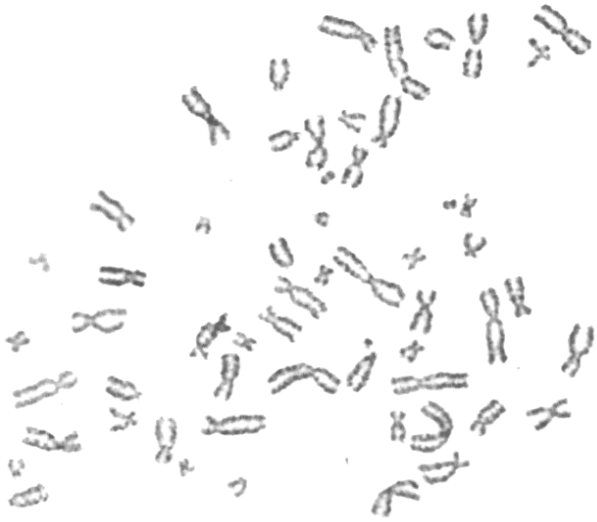

Fig.2 Results of chromosome G banding analysis. The chromosome did not extend entirely in the prophase of cell division. The numbers could be counted, but the bands could not be recognized (Giemsa stain, ×1000).

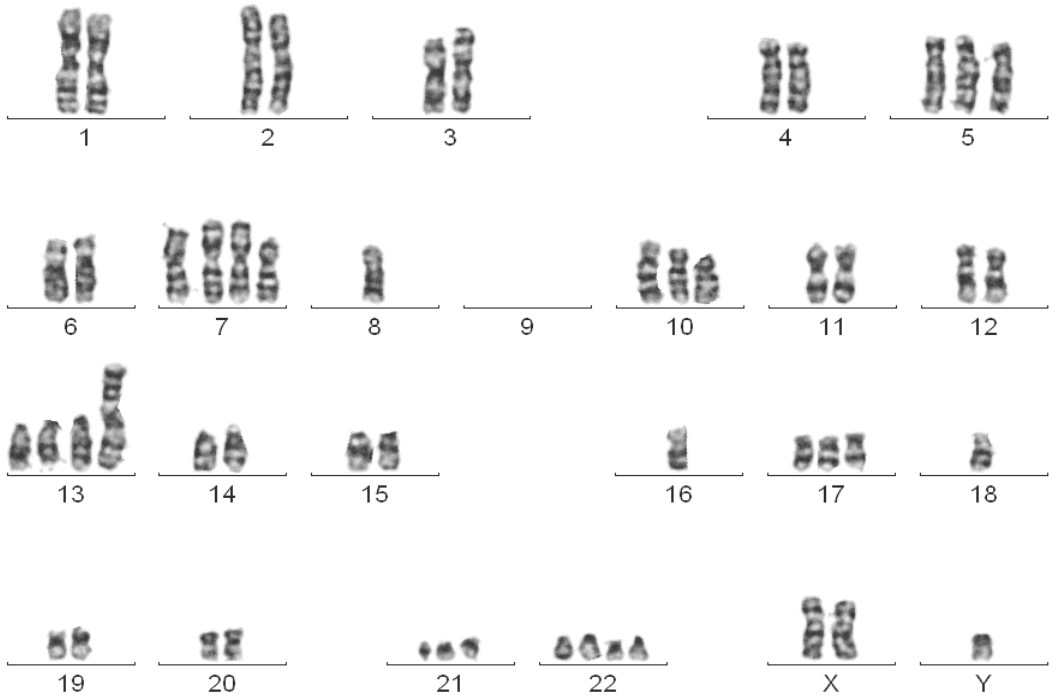

Fig.3 Results of Karyotype analysis. The chromosome extended entirely in the metaphase of cell division. The numbers could be counted, and the bands could be recognized clearly (Giemsa stain, ×1000).

3.4 Establishment of bone metastasis model

After inoculated by SPC-A-1BM cells *via* left ventricle of the BNX mice, all the eight mice developed bone metastatic lesion. The 111-MBq ^{99m}Tc-MDP group demonstrated a characteristic appearance with osteoclastic destruction (Fig.4a). Metastatic lesions were seen in the mandible, upper limb and thoracic vertebrae, whereas the other ^{99m}Tc-MDP groups or the X-ray group did not exhibit the bone metastasis clearly (Fig.4b). On H&E staining of the bone metastasis sites detected by SPECT, the skeletal metastasis of pulmonary adenocarcinoma in the mice showed the characteristic cell morphology typical for this tumor entity in the thoracic vertebrae (Fig.4c).

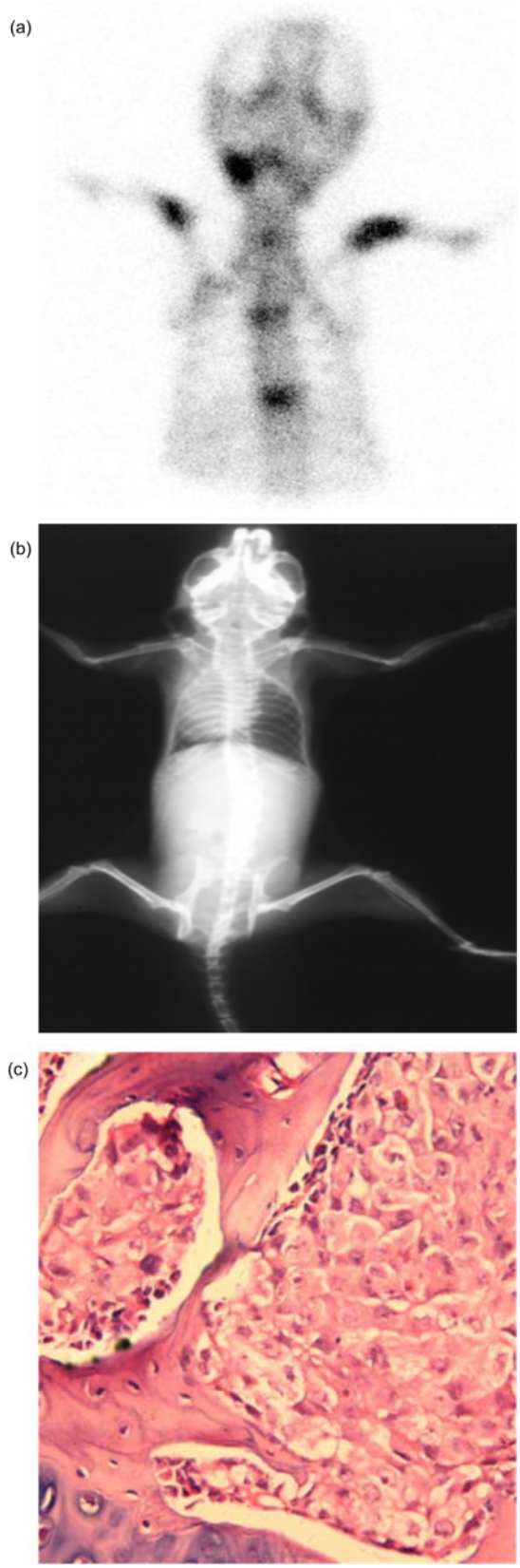

Fig.4 Images of a BNX mouse inoculated by SPC-A-1BM cells 14 days later. BS after iv injection of 111MBq ^{99m}Tc-MDP (a), X-ray image of the BNX mouse on the same day (b) and histological features of the thoracic vertebrae metastasis (H.E. × 100) (c).

4 Discussion

BS is a sensitive and efficient method to measure metabolic activity of the entire skeleton. ^{99m}Tc-MDP is well established for screening most bony metastasis disease. It is more sensitive than XR for identifying bone metastases from carcinoma[1,3,13]. The pinhole collimation SPECT has very high resolution of ≤ 2 mm (FWHM) at 3 cm from the collimator, and high sensitivity of ≥ 216 counts·s^{-1}·MBq^{-1} at 3 cm from the collimator in imaging small object[19]. The pinhole collimator (d=1mm) used in this study provided a high resolution image. It is noticeable that a relatively clear image of nearly each rib can be detected (Fig.4a).

Chromosomal rearrangements are the best-characterized end point of radiation-induced genomic instability, and many of the rearrangements described are similar to those found in human cancers[15]. Multiple chromosomal abnormalities have been described in a variety of human cancers[20]. The pattern of abnormalities varies greatly between malignancies, ranging from simple balanced rearrangements to complex abnormalities affecting both chromosome structure and number including cytogenetically visible changes such as chromosome losses or gains, translocations and deletions[21].

Lorimore *et al*[22] exposed cells to alpha particles, but interposed a grid between some of the cells and the alpha particle source so that the surviving population consisted predominantly of cells not traversed by radiation. Their data clearly demonstrated that chromosomal instability could be induced in the non-irradiated cells indicating unexpected interactions between irradiated and non-irradiated cells. Subsequent studies have demonstrated that non-targeted or bystander-like effects may play a significant role in induced genomic instability[23,24].It is not surprising that inadequate dose of isotope in BS may induces chromosomal abnormalities of the cancer cells by direct effects or bystander-like effects.

There is a need to develop an animal model in order to study the role of ^{99m}Tc-MDP involved with sensitivity of images and susceptibility of the chromosome of the tumor cell. Murine

adenocarcinomas have been shown to resemble those found in humans with respect to cell of origin, structure of the tumor and a strong genetic influence on susceptibility[25]. Our results indicate for the first time that we have developed a novel, reproducible mouse model of bone metastasis by using human pulmonary adenocarcinoma cell line (SPC-A-1BM). And it is confirmed by histopathologyical study (Fig.4c). This model allows us to investigate the role of ^{99m}Tc-MDP in the process of establishment and will, therefore, be instrumental in studying many aspects of tumor cell biology, including organ-selective metastasis and tumor angiogenesis.

Mouse chromosomes 1 and 15 were the most common region of chromosomal alteration in the mouse lung tumor cell strains and were associated with an invasive phenotype[26]. Deletions of chromosome 15 were observed only in the low invasive cell strains, further indicating the potential importance of this chromosomal gain for cell invasion [26]. In this study, the 1 and 15 chromosome number did not change (Fig.3). Extra copies of human chromosomes 1 and 8 are associated with an invasive phenotype in human pulmonary adenocarcinoma[27,28]. Besides, in the present study, chromosomes 3, 7 and 17 were used to evaluate chromosomal damage. Cytogenic studies have revealed frequent alterations in a variety of chromosomes in bladder cancer, including chromosomes 1, 3, 7, 9, 11 and 17[29,30]. But there are seldom paralleled reports about lung cancer. Our results indicated that the number of chromosome 8 decreased and chromosome 7 and 17 increased. Whether the decreased or increased copy number is associated with a metastasis phenotype of mouse lung cancer shall be investigated in future.

The study indicates that ^{99m}-Tc-MDP bone scintigraphy with pinhole SPECT plays a key role in detecting bone metastasis of human pulmonary adenocarcinoma in animal model. This provides another way to evaluate the bone metastasis in animal model and will simplify the dose-administration in laboratory. Clearly, there is considerable room for improvement and this will be achieved by further investigations.

References

1 Husband J E S, Reznek R H. Imaging in Oncology. Oxford, U.K.: Isis Medical Media Ltd., 1998, 765-787.

2 Blake G M, Park-Holohan S J, Cook G J, *et al.* Semin Nucl Med, 2001, **31:** 28-49.

3 Hamaoka T, Madewell J E, Podoloff D A, *et al.* J Clin Oncol, 2004, **22:** 2942-2953.

4 Fogelman I. Eur J Nucl Med, 1982, **7:** 506-509.

5 Arveschoug A K, Bertelsen H, Vammen B. Clin Nucl Med, 2002, **27:** 249-254.

6 Spanu A, Falchi A, Manca A, *et al.* J Nucl Med, 2004, **45**: 40-48.

7 Profanter C, Gabriel M, Wetscher G J, *et al.* Surg Today, 2004, **34:** 493-497.

8 Carlier T, Oudoux A, Mirallié E, *et al*. Eur J Med Mol Imaging, 2008, **35:** 637-643.

9 Jemal A, Travis W D, Tarone R E, *et al*. Int J Cancer, 2003, **105:** 101-107.

10 Rybak L D, Rosenthal D I. Q J Nucl Med, 2001, **45:** 53-64.

11 Schirrmeister H, Guhlnamm A, Kotzerke J, *et al*. J Clin Oncol, 1999, **17:** 2381-2389.

12 Yoneda T, Williams P J, Hiraga T, *et al*. J Bone Miner Res, 2001, **16:** 1486-1495.

13 Kang Y, Siegel P M, Shu W, *et al*. Cancer Cell, 2003, **3:** 537-549.

14 Bacher K, Thierens H M. Nucl Med Commun, 2005, **26:** 581-586.

15 Huang L, Snyder A R, Morgan W F. Oncogene, 2003, **22:** 5848-5854.

16 Yang S F, Dong Q G, Yao M, *et al*. Cancer, 2006, **26:** 1059-1063 (In Chinese).

17 Zietman A L, Sugiyama E, Ramsay J R, *et al*. Int J Cancer, 1991, **47:** 755-759.

18 O′Sullivan J M, Cook G J. Q J Nucl Med, 2002, **46:** 152-159.

19 Li J, Jaszczak R J, Greer K L, *et al*. Phys Med Biol, 1994, **39:** 165-176.

20 Mitelman F. Mutat Res, 2000, **462:** 247-253.

21 Lengauer C, Kinzler K W, Vogelstein B. Nature, 1998, **396:** 643-649.

22 Lorimore S A, Kadhim M A, Pocock D A, *et al.* Proc Natl Acad Sci U S A, 1998, **95:** 5730-5733.

23 Watson G E, Lorimore S A, Macdonald D A, *et al*. Cancer Res, 2000, **60:** 5608-5611.

24 Lorimore S A, Coates P J, Scobie G E, *et al*. Oncogene, 2001, **20:** 7085-7095.

25 Malkinson A M. Cancer Res, 1992, **52(9 Suppl):** 2670s-2676s.

26 Sargent L M, Ensell M X, Ostvold A C, *et al*. Toxicol Appl Pharmacol, 2008, Feb 8. [Epub ahead of print].

27 Lu Y J, Dong X Y, Shipley J, *et al*. Lung Cancer, 1999, **23:** 61-66.

28 Kubokura H, Tenjin T, Akiyama H, *et al*. Ann Thorac Cardiovasc Surg, 2001, **7:** 197-203.

29 Yu D S, Chen H I, Chang S Y. Urol Int, 2002, **69:** 129-135.

30 Sarosdy M F, Schellhammer P, Bokinsky G, *et al*. J Urol, 2002, **168:** 1950-1954.