synovitis and and giant cell tumor of tendon sheath express features of osteoclasts. Am J Pathol 150: 1383-92,1997

11. Young JM, Hudacek AG: Experimental production of pigmented villonodular synivitis in dogs. Am J Pathol 30:799-811,1954

12. Sigh R, Grewal DS, Chakravarti RN: Experimental production of pigmented villonodular synovitis in the knee and ankle joints of rhesus monkey. J Path 98:137-142,1969

13. Somerhausen NSA, Flrcher CDM: Diffuse-tupe giant cell tumor. Clinicopathologic and immunohistochemical analysis of 50 cases with extraarticular disease. Am J Surg Pathol 24:479-492,2000

14. Abdul-Karim FW, El-Naggar AK. Joyce MJ, Makley JT, Carter JR. Diffuse and localized tenosynovial giant tumor and pigmented villonodular synovitis: a clinicopathological and flow cytometric DNA analysis. Hum Pathol 23:729-35,1992

15. Fletcher JA, Henkle C, Atkins L, Rosenberg AE, Morton C. Trisomy 5 and trisomy 7 are nonrandom aberrations in pigmented villonodular synovitis: confirmation of trisomy 7 in uncultured cells. Genes chromosomes cancer 4:264-266,1992

16. Schumacher HR, Lotke P, Athreya B, Rothfuss S. Pigmented villonodular synovitis:light and electron microscopic studies. Semin Arthrits Rheum 12:32-43,1982

17. Ghadially FN, Lalonde J-M A, Dick CE. Ultrastructure of pigmented villonodular synovitis. J Path.127:19-27,1978

18. Wyllie JC. Stromal cell reaction of pigmented villonodular synovitis: an electron

microscopic study. Arthritis Rheum. 12(3):205-214,1969

19. Maximov V, Martynenko A, Hunsmann G, Tarantul V. Mitochondrial 16S rRNA gene encodes a functional peptide, a potential drug for Alzheimer's disease and target for cancer therapy. Medical Hypothese 59(6):760-673,2002

20. Guo B, Zhai,D, Cabezas E, Welsh K, Niourain S, Tatterthwait A,Reed JC.: Humanin peptide suppresses apoptosis by interfering with Bax activation. Nature,online 4 May,2003

21. Chomczynski P, Sacci N. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chlorform extraction. Anal Biochem162:156-9,1987

22. Majima JH, Oberley TD, Fukukawa K, Mattson MP, Yen HC, Szweda LI, St. Clair DK. Prevention of mitochondrial injury by manganses superoxide dismutase reveals a primary mechanism for alkaline-induced cell death. J Biol Chem 273:8217-24,1998

23. Motoori, S., Majima, H.J., Ebara, M., Kato, H., Hirai, F., Kakinuma, S., Yamaguchi, C., Ando, K., Ozawa, T., Nagano, T., Tsujii, H., and Saisho, H.: Overexpression of mitochondrial manganese superoxide dismutase protects against radiation-induced cell death in the human hepatocellular carcinoma cell line, HLE. Cancer Res. 61:5382-5388, 2001.

24. Peng G, Taylor JD, Tchen TT. Increased mitochondrial activites in pigmented (melanized) fish cells and nucleotide sequence of mitochondrial large rRNA. Biochem Biophys Res Commun 189(1):445-9,1992

25. Baserga SJ, Linnenbach AJ, Malcolm S. Polyadenylation of a human mitochondrial ribosomal RNA transcript detected by molecular cloning. Gene 35:305-312,1985

26. Tarantul V, Nikolaev A, Hannig H. Detection of abundantly transcribed genes and gene translocation in human immunodeficiency virus-associated non-Hodgkin's lymphoma. Neoplasia 3:132-142,2001

27. Penta J, Johnson FM, Wachsman JT, Copeland WC. Mitochondrial DNA in human malignancy. Mutat Res 488:119-133,2001

28. Hashimoto Y, Niikura T, Tajima H, Yasukawa T, Sudo H, Ito Y, Kita Y, Kawasumi M, Kouyama K, Doyu M, Soube G, Koide T, Tsuji S, Lang J, Kurokawa K, Nishimoto I. A rescue factor abolishing neural cell death by a wide spectrum of familial Alzheimer's disease. PNAS 98(11):6336-6341,2001

29. Hashimoto Y, Ito Y, Niikura T. Mechanism of neuroprotection by a novel rescue factor Humanin from Swedish mutant amyloid precursor protein. Biochem Biophy Res Commun 283:460-468,2001

30. Yu W, Sanders BG, Kline K.:RRR-alpha-tocopheryl succinate-induced apoptosis of human breast cancer cells involved bax translocation to mitochondria. Cancer Res 63(109:2483-91,2003

31. Nakazawa Y, Kamijyo T, Koike K, Noda T.: ARF tumor suppressor induces mitochondria-dependent apoptosis by modulation of mitochondrial Bcl-2 family proteins. J Biol Chem ,in press, May 9, 2003

32. Muirden KD. The anemia of rheumatoid arthritis: the significance of iron deposits in the synovial membrane. Aust Ann Med 2:97-104,1970

33. Brunk UT, Terman A. The mitochondrial-lysosomal axis theory of aging: accumulation of damaged mitochondria as a result of imperfect autophagocytosis. Eur J Biochem :269(8):1996-2002,2002

34. Morris CJ, Wainwright AC, Steven MM. The nature of iron deposits in haemophilic synovitis – an immunohistochemical ultrastructural and x-ray microanalytical study. Virchows Arch [Cell Pathol] 404:75-85,1984

35. Docken WP. Pigmented villonodular synovitis: a review with illustrative case reports. Semin Arthritis Rheum 9:1-22,1979

36. Wixom RL, Prutkin L, Munro HN. Hemosiderin: nature, formation, and significance. Intl. Rev. Exper. Pathol 22:193,224,1980

37. Chamberlain MA, Petts V, Gollins E. Transport of intravenously injected ferritin across the guinea-pig synovium. Ann Rheum Dis 31:493-9,1972

38. McCord JM, Roy RS. The pathophysiology of superoxide; roles in inflammation and ischaemia. Can J Physiol Pharmacol 60:1346-52,1982

39. Halliwell B, Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 219:1-4,1984

40. Panduri V, Weitzman SA, Chandel N, Kamp DW. The mitochondria-regulated death pathway mediates asbestos-induced alveolar epithelial cell apoptosis. Am J Respir Cell Mol Biol ;28(2):241-8,2003

41. Gutteridge JM, Halliwell B, Treffry A, Harrison PM, Blake DR. Effect of ferritin containing

fractions with different iron loading on lipid peroxidation. Biochem J 209:557-60,1983

42. Crichton RR. Interreaction between iron methabolism and oxygen activation. In Oxygen free radicals and tissue damage. Amsterdam: Excerpta Medica:57-76,1979

43. Morris CJ, Blake DR, Wainwright AC, Steven MM. Relationship between iron deposits and tissue damage in the synovium: an ultrastructural study. Ann Rheum Dis 45:21-6,1986

Mitochondrial	
16S rRNA	30
12S rRNA	5
Homosapiens Tomoregulin mRNA	2
Homosapiens ARFGAP 1 protein mRNA	1
Mitochondrial proteolipid 68 MP homology	1
Inflammation	
β2-microglobulin mRNA	1
TGF-β mRNA	1
Fibrogenolysis	
Arg/serpin 1 plasminogen activator-inhibitor 2 mRNA	1
Homosapiens similar to serine proteinase mRNA	
Homosapiens similar to serine/arginin repetitive matrix mRNA	1
Iron metabolism	
Ferritin light chain mRNA	1
Cartilage degradation	
Homosapiens dihydropyrimidinase mRNA	1
Homosapiens osteopontin mRNA	1

Neoplastic	
L-plastin mRNA	1
Others	
Eukaryotic translation elongation factor mRNA	2
Homosapiens Nef-associated factor mRNA	1
Unknown	2
Total	68 clones

Table1. Highly expressed genes in PVNS compared with RA.

Figure Legends

Fig.1. The sequences encoded within the 16S rRNA region with poly A tail. The cDNA fragments were aligned with the 16S rRNA region of the mitochondrial gene and the correlating humanin mRNA sequence. Southern colony hybridizations repeated these sequences in a total of three rounds independently. Oblique bars show the digestion sites by Rsa I and upward diagonal bar shows the region of humanin CDS. Although there are nine types of sequences with poly A tail within this region, only the type 9 sequence was identical to the previously reported mRNA encording humanin peptide.

Fig.2. Northern blot analysis of mRNAs expressed by synovial cells from PVNS, RA and OA patients. Total RNA (168ng) was subjected to electrophoresis in a 1.0% agarose gel containing formaldehyde, transferred to nylon membrane, and probed with [^{32}P-dCTP] labeled cDNA (type 9; Table 1). Another cDNA (type 3) encoded in the 16S rRNA region was also used in Northern blotting and the expression level and size were same as those using type 9 cDNA(data not shown). Humanin genes were strongly expressed in diffuse type PVNS, but barely detected in nodular type PVNS, RA, or OA. The size of the expressed major message was ~1.6 kb and the other messages were ~1 kb, which correspond to the results of previous report by Hashimoto et al. (30)

Fig 3. The expression of genes encoded in mitochondria other than humanin genes.

Total RNA was extracted from synovial cell of 5 patients with PVNS, 3 with RA and 3 with OA and NADH dehydrogenase, ATPase 6, Cytocrome c, Cytocrome b and GAPDH mRNA levels were analyzed by semiquantitative RT-PCR. The levels of expression of these genes in PVNS were not increased in other types of arthritis, indicating that humanin gene was selectively expressed in mitochondrial genes in PVNS.

Fig 4. The expression of humanin peptide in synovial cells from diffuse type PVNS. Twenty μg of protein from synovial cell lysates were subjected to SDS-PAGE on a 5-20% gradient gel. Rabbit anti-humanin polyclonal antibody was used for Western blotting. Synthesized peptide, which was used as antigen to produce rabbit anti-humanin polyclonal antibody, was used as a standard and rabbit IgG was used as a negative control.

Fig. 5. The synovial tissue from diffuse type PVNS was fixed with 4% formaldehyde in PBS. The specimens were stained with anti-humanin antibody, followed by Alexa 488 goat anti-rabbit IgG and photographed with a fluorescent microscope(40 x). (a). Most positive cells (green) were distributed in deep layer with hemosiderin deposit. (c). Negative control of the continuous section.(b) and (d). backgrounds for a or c, respectively.

Fig. 6. The relationship between humanin peptide expression and mitochondria. Isolated hemosiderin-containing synovial cells were double-stained with anti-humanin antibody and anti

HSP 60 antibody as first antibodies, followed by goat anti-rabbit IgG and donkey anti-goat IgG as second antibodies(400 x). (a) Hemosiderin was deposited unequally throughout the cytoplasm and (d) humanin was dominantly distributed in the mitochondria around the siderosome (yellow). (b). single anti-humanin antibody staining (red). (c.) single anti-HSP 60 antibody staining (mitochondrial staining;green).

Fig. 7. Elecron micrograph of synovial cells from diffuse type PVNS. Most of the electron dense iron deposits were observed within the siderosomes. Some electron dense iron deposits were observed within mitochondria(arrows). Mitochondrial membrane debris with electron dense deposits was observed within the siderosome as an autophagosome (left arrow) (a). Some of normal mitochondria (arrows) also were scattered throughout the cytoplasm (b). (Magnification 19000 X)

Fig. 8. Electron microscopic immunohistochemistry of synovial cells from diffuse type PVNS. In some of the siderosomes, particles of colloidal-gold, were precipitated to the debris adjacent to electron dense iron (a). These results demonstrate that humanin peptide is present within the debris that are phagocytosed into the siderosome. Negative control for immunohistochemistry (b). (Magnification 29000 X)

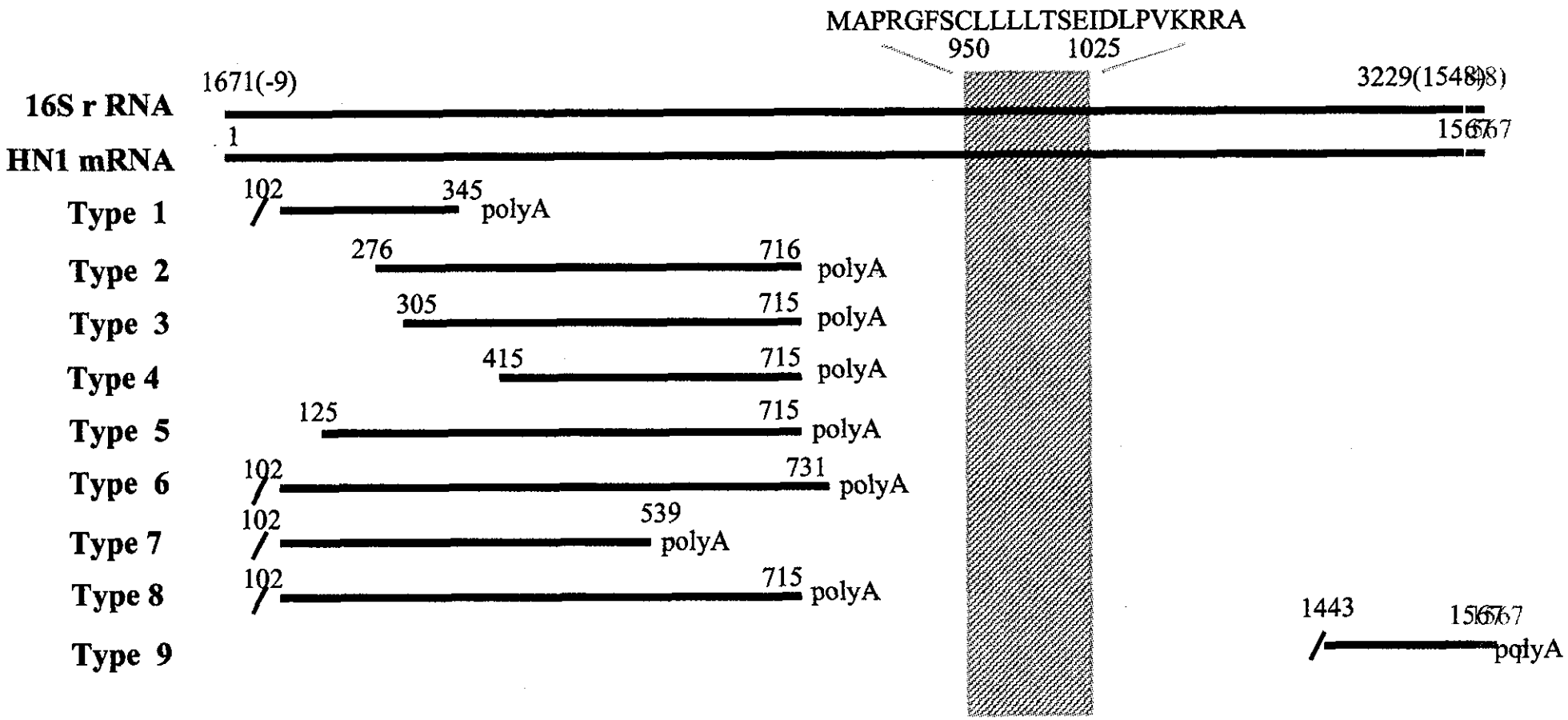


Fig.1. Kosei Ijiri

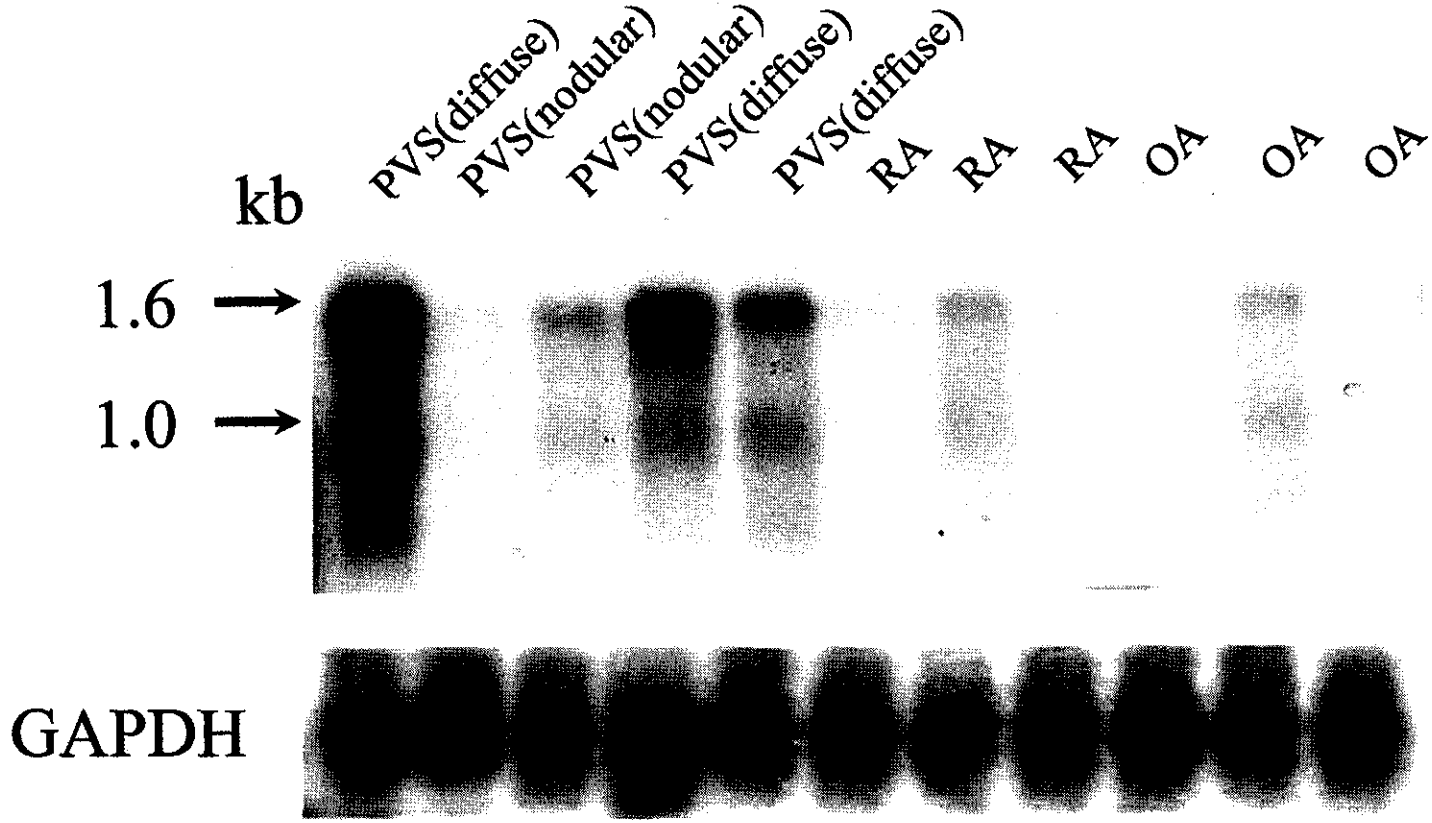


Figure 2. Kosei Ijiri

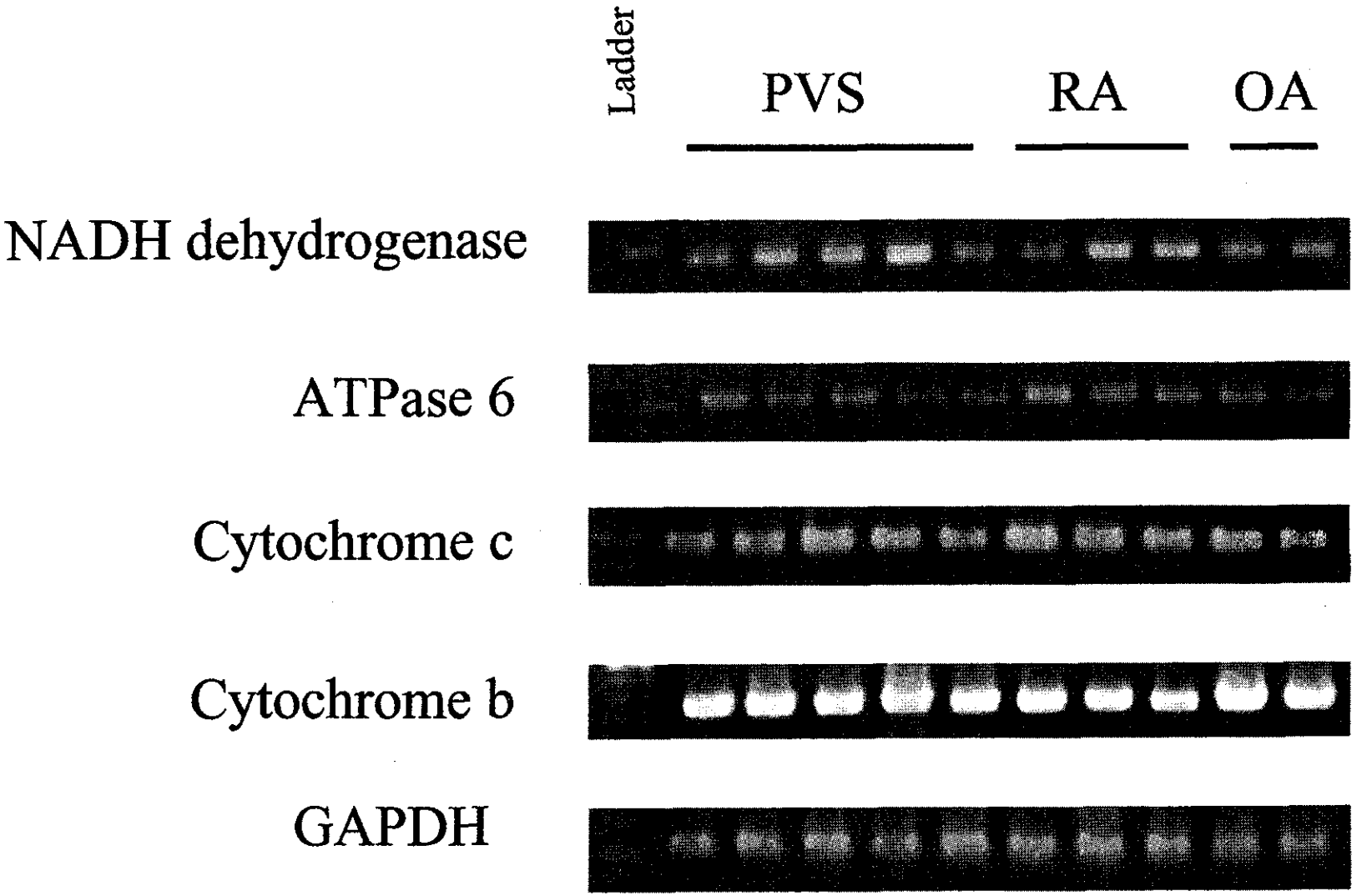


Figure 3. Kosei Ijiri

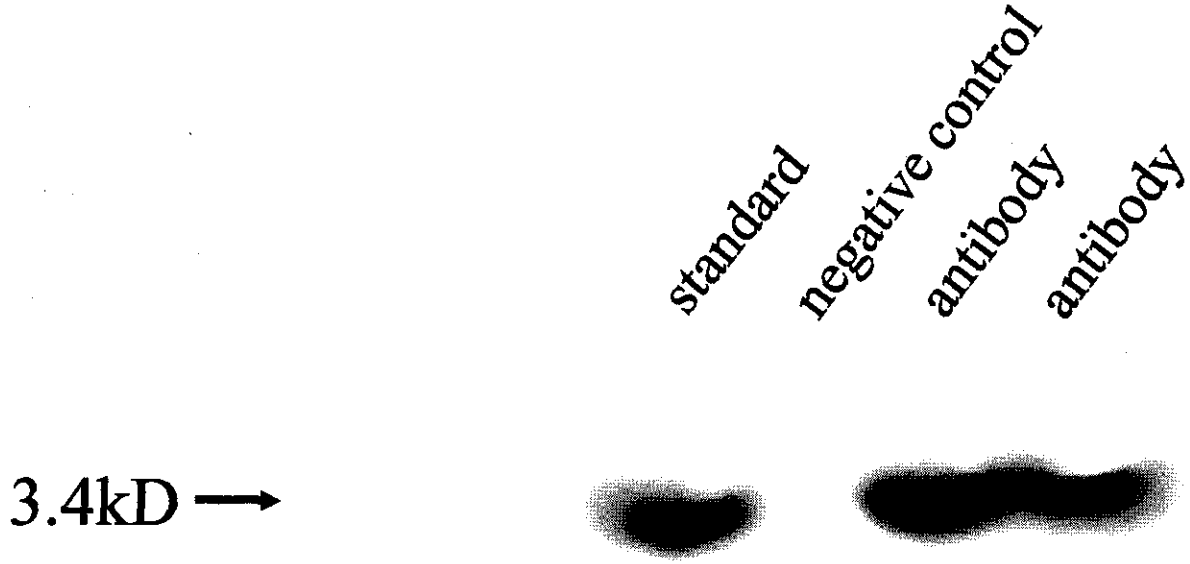


Figure 4. Kosei Ijiri

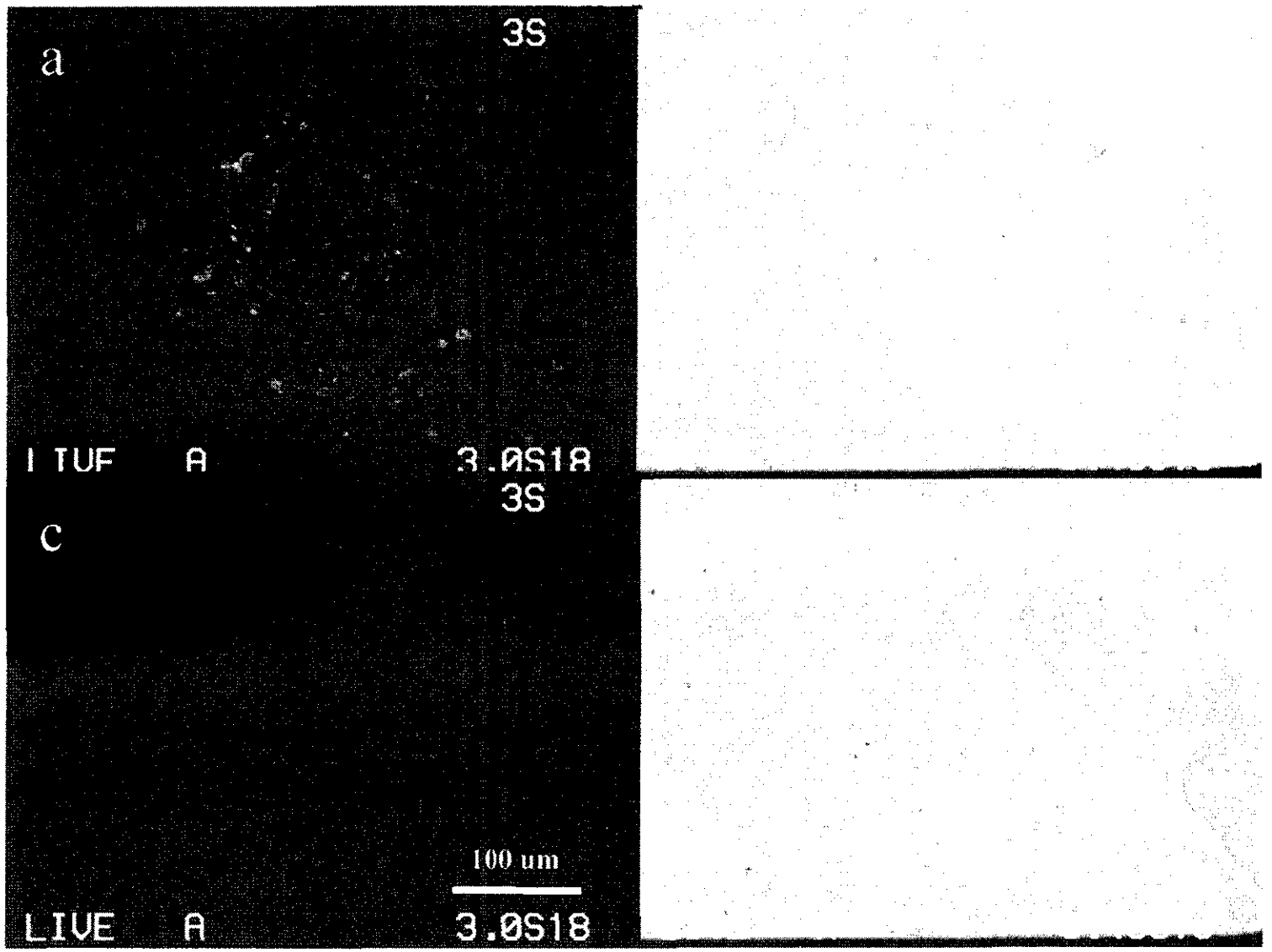


Figure 5. Kosei Ijiri

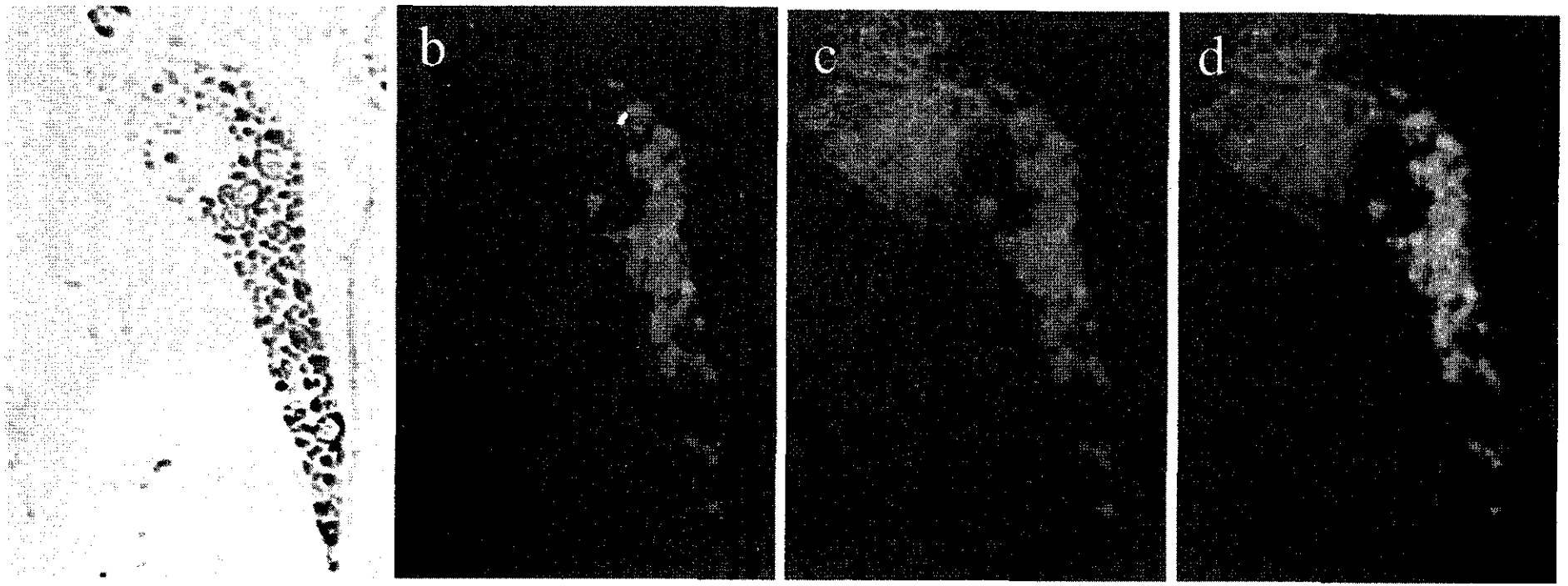
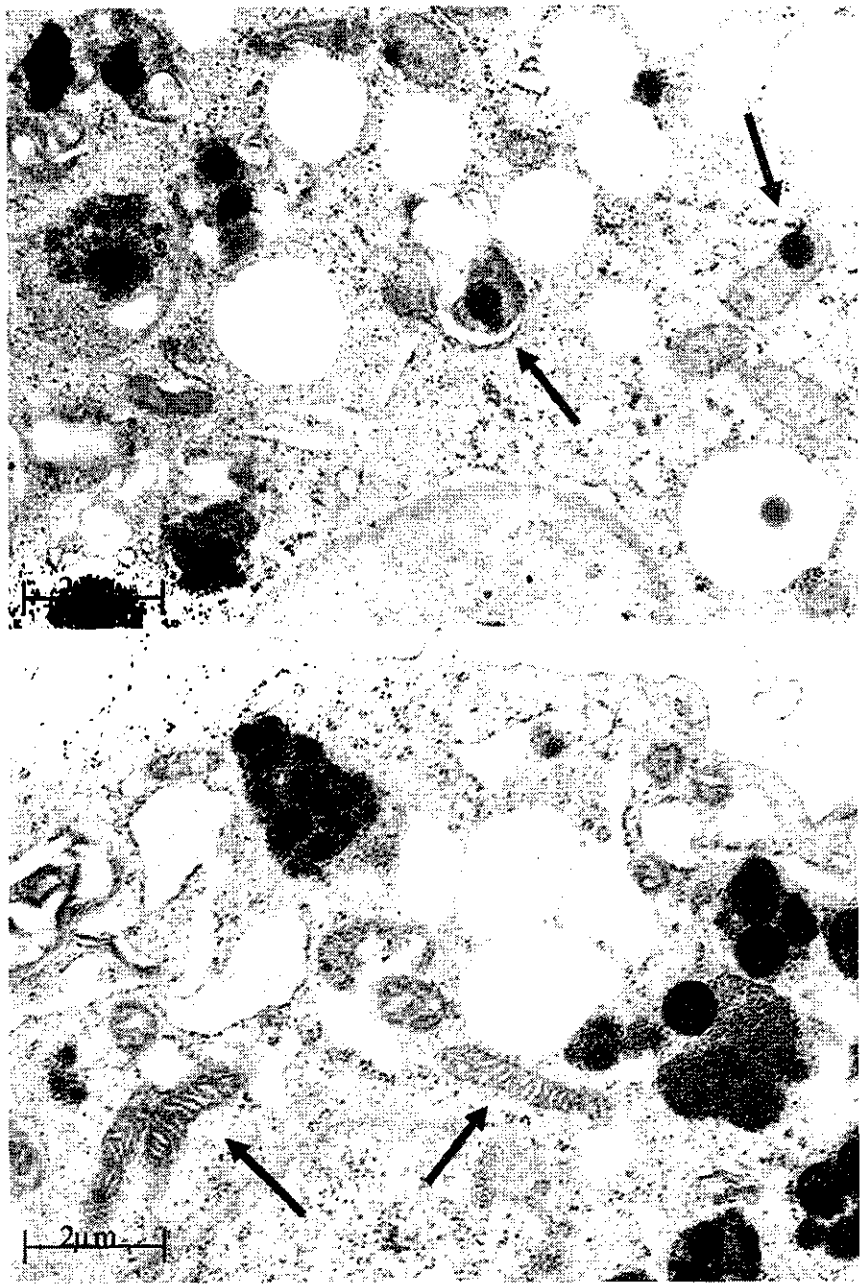


Figure 6. Kosei Ijiri



a

b

Figure 7. Kosei Ijiri

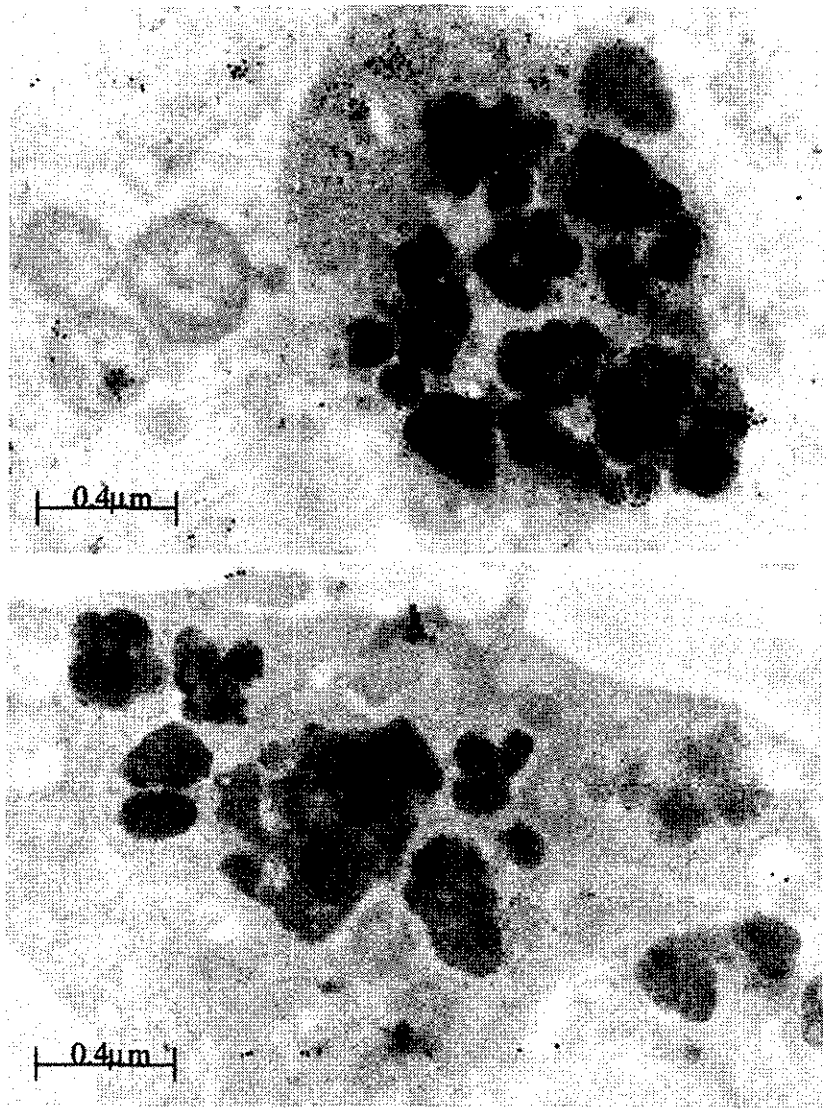


Figure 8. Kosei Ijiri

Lung cancer death rates by smoking status: Comparison of the Three-Prefecture Cohort study in Japan to the Cancer Prevention Study II in the USA

Tomomi Marugame,[1,10] Tomotaka Sobue,[1] Hiroshi Satoh,[2] Shoko Komatsu,[4] Yoshikazu Nishino,[3] Haruo Nakatsuka,[5] Tomio Nakayama,[6] Takaichiro Suzuki,[6] Toshiro Takezaki,[7] Kazuo Tajima[8] and Suketami Tominaga[9]

[1]Statistics and Cancer Control Division, Research Center for Cancer Prevention and Screening, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104–0045, [2]Environmental Health Sciences, [3]Division of Epidemiology, Department of Public Health and Forensic Medicine, Tohoku University Graduate School of Medicine, 2–1 Seiryo-machi, Aoba-ku, Sendai 980–8575, [4]Faculty of Physical Education, Sendai College, 2-2-18 Funaokaminami, Shibata-machi, Shibata, Miyagi, 989–1693, [5]Miyagi University, 1 Gakuen, Taiwa-cho, Kurokawa, Miyagi, 981–3298, [6]Department of Cancer Control and Statistics, Osaka Medical Center for Cancer and Cardiovascular Diseases, 1-3-3 Nakamichi, Higashinari-ku, Osaka 537–8511, [7]Department of International Island and Community Medicine, Kagoshima University Graduate School of Medical and Dental Science, 8-35-1 Sakuragaoka, Kagoshima 890–8520, [8]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, 1–1 Kanokoden, Chikusa-ku, Nagoya 464–8681, [9]Aichi Comprehensive Health Science Center of the Aichi Health Promotion Foundation, 1–1 Gengoyama, Morioka, Higashiura, Aichi 470–2101, Japan

(Received August 11, 2004/Revised November 15, 2004/Accepted Novermber 24, 2004/Online publication 17 February, 2005)

Cigarette smoking is an established risk factor for lung cancer. However, the magnitude of the relative risk (RR) on lung cancer mortality in relation to cigarette smoking is reported to be lower in Japan than in Western countries. We investigated whether this discrepancy could be explained by differences in the exposure to cigarettes smoked, by differences in sensitivity to smoking, or by differences in lung cancer mortality among non-smokers. We examined the 10-year follow-up data on 88 153 participants in a Japanese population-based prospective study conducted in three prefectures. Data used as a Western counterpart was retrieved from a published report of the US Cancer Prevention Study (CPS)-II. Although there was a significant increased risk of lung cancer death among current smokers compared with non-smokers, the observed RR in the Three-Prefecture Study were much lower than RR reported in the CPS-II. Lung cancer mortality of our Japanese sample was lower among current smokers and higher among non-smokers regardless of age and sex. Current smokers in our sample had initiated smoking at an older age and smoked fewer cigarettes per day for shorter durations than those in the CPS-II sample. The Poisson regression model (controlling for age, number of cigarettes smoked per day and duration of smoking) showed that male current smokers in our sample had a lower risk of lung cancer compared with those in the CPS-II sample (rate ratio 0.34 [95%CI 0.27–0.43]). These findings might explain why Japanese risks of lung cancer are lower than those observed in Western countries. (*Cancer Sci* 2005; 96: 120–126)

Numerous epidemiological studies have consistently reported smoking as a risk factor for lung cancer. Three prospective studies[1–3] and several case-control studies[4–6] in Japan have shown that the magnitude of the relative risk (RR) associated with cigarette smoking is lower than those in Western countries.[2] For example, in the Six-Prefecture Study[3] and the Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC),[1] the RR of lung cancer death among smokers compared to non-smokers was estimated at 4.5 for men, whereas the RR for men ranged from 11.6 to 23.2 in prospective studies conducted in the USA[7–9] and the UK.[10] For women, the RR were 2.3 in the Six-Prefecture Study[3] and 3.6 in the JACC study,[1] while corresponding RR ranged from 2.7 to 12.8 in the USA.[7,9] The first aim of this study was to verify these figures by evaluating lung cancer death and smoking habits with a new large-scale, population-based prospective survey (The Three-Prefecture Cohort Study), conducted in three prefectures in Japan.

The RR expresses a single summary estimate of the effects of smoking on lung cancer. However, the RR is computed by simply dividing the death rate among smokers by that among non-smokers. For a better understanding of the reasons for the lower RR of lung cancer among the Japanese, it would be more accurate to compare the death rates by smoking status. Furthermore, exposure levels to smoking might account for differences in the risk of lung cancer between Japanese and Western current smokers. It is well known that lung cancer risk depends on the amount, duration, and initiation age of smoking. Thus, to determine the reason for the lower RR associated with smoking in Japanese subjects, it is also important to compare the exposure levels to smoking as well as the lung cancer death rates between Japanese and Western subjects.

The second aim of this study was to compare death rates by smoking status and smoking exposure levels with published data from a large American prospective sample, the Cancer Prevention Study II (CPS-II),[9] which began at nearly the same time as the Three-Prefecture Cohort Study (1982). Finally, we examined whether any discrepancy in the RR of lung cancer between the studies could be explained by the difference in death rates due to smoking status (i.e. non-smokers vs smokers) and smoking exposure level between the Japanese and the US samples.

Materials and Methods

Study population. The Three-Prefecture Cohort Study collected data from February 1, 1983 to November 1, 1985, in selected areas of three prefectures in Japan: Miyagi, Aichi, and Osaka. The study areas of each prefecture included six areas of a city and two towns in Miyagi Prefecture, five elementary school districts in one area of a city and two areas of a city in Aichi Prefecture, and three towns in Osaka Prefecture. An additional study cohort was sampled in December 1, 1990, in one city in the Osaka Prefecture. The study population included all persons aged 40 years or older, who resided in the study areas according to each town's residential registry. A self-administered questionnaire was distributed to 130 839 persons, and 108 774 (50 544 men and 58 230 women) of them responded (83.1%). We then excluded individuals under 40 years (one man and one woman) and over 80 years of age (1 427 men and 2 465 women), any who

[10]To whom correspondence should be addressed. E-mail: tmarugam@gan2.res.ncc.go.jp

 doi: 10.1111/j.1349-7006.2005.00013.x

moved out before the start of the follow up (five men and three women), and any whose information on smoking status at enrollment was incomplete (4 660 men and 12 059 women). After exclusion of these individuals, 44 451 men and 43 702 women remained in the analysis. This study was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

Follow up. Information on whether each subject was still alive and living in the same location was obtained from residential registries. If the subject had died, we then searched the population-based cancer registry in each prefecture and ascertained whether they had died from lung cancer. Sites of any cancers were coded using the International Classification of Disease and Injuries–ninth revision (ICD-9), except for one city in Osaka where the ICD 10th revision was used. Study subjects were followed for 10 years in each area. Therefore, the end of the study period varied from January 31, 1993 to October 31, 1995 (and February 28, 2000 for the one city in the Osaka Prefecture) according to the dates of enrollment. During the follow up, 8 836 (15.6%) individuals moved out of the study areas.

Smoking Information. At enrollment, study participants completed a self-administered questionnaire, including demographic information such as sex, date of birth, and smoking habits. The smoking habits questions were the same in each study area, except for one town in the Osaka Prefecture. All participants were asked: 'Do you smoke?' Response categories included: (1) yes; (2) smoked but quit; and (3) never smoked. We defined participants who chose response (1) as current smokers; those who chose response (2) as former smokers; and those who chose response (3) as non-smokers. For one city in the Osaka Prefecture, the response categories were: (1) yes (smoking every day); (2) yes, but occasionally; (3) smoked, but quit; and (4) never smoked. We defined participants who chose response (1) and (2) as current smokers, those who chose response (3) as former smokers, and those who chose response (4) as non-smokers.

The ages at initiation of smoking and the average number of cigarettes smoked per day for current and former smokers were obtained. The number of years of smoking that current smokers had smoked prior to enrollment was calculated by subtracting the age at initiation of smoking from the age at enrollment. Pack-years were defined as the number of years of smoking multiplied by the number of packs of cigarettes per day.

Cancer Prevention Study II. The CPS-II[9] is a prospective cohort study, conducted by the American Cancer Society (ACS). It was selected as the Western counterpart to our Japanese prospective cohort study because it contained detailed data on lung cancer mortality by sex, age group and smoking status, as well as data on smoking patterns of current smokers by sex and age group. The CPS-II data for the comparison were retrieved from the Smoking and Tobacco Control Monograph no. 8. Study participants were friends, neighbors, and acquaintances of ACS volunteers. Approximately 1.2 million men and women were enrolled in 1982. Enrollment included all household members 30 years of age or older if at least one family member was 45 years of age or older. Study participants completed an initial questionnaire including smoking habits and other lifestyle factors. The vital status of study participants was determined through personal inquiry by the volunteers. The underlying cause of death was obtained through death certificates. During the 6-year follow up of 711 363 current cigarette smokers and lifelong non-smokers, 3 229 died of lung cancer.

Statistical Analysis. Person years during the follow-up were counted from the date of enrollment into the study until the date of death, migration from the study areas, or the end of the study period, whichever came first. The RR was estimated with a Cox proportional hazards model with adjustments for age (continuous variable) and prefecture. Non-smokers were used as a reference category. A dose–response relationship among current smokers was examined in terms of the number of pack-years.

Using data from the CPS-II, we compared the baseline data on smoking patterns among current smokers and the follow-up data on lung cancer deaths among non-smokers and current smokers. Follow-up data were restricted to the first 6 years, the duration of the CPS-II. The mean number of cigarettes smoked per day and the mean number of years of smoking were calculated within the 5-year age groups fixed at the baseline. The age-adjusted number of cigarettes smoked per day and the age-adjusted number of years of smoking was obtained by directly standardizing to the combined distribution of age groups of the Japanese and US cohorts. Because the mean age at initiation of smoking among the CPS-II subjects was provided as 10-year birth cohorts, we calculated mean age of initiation in the Japanese study in the same way.

Sex- and age-specific death rates of lung cancer (per 100 000) were computed for non-smokers and current smokers. Calculation of the number of person years at risk was based on attained age. To compare the death rates of the Japanese and US cohorts, cumulative death rates between 40 and 84 years were presented. Rate ratios of the Japanese cohort to US cohort were calculated by using a Poisson regression model.

Lung cancer death rates were computed for male current smokers, stratified by the duration of smoking and the number of cigarettes smoked per day. Because of limited CPS-II data, only subjects who smoked 20 or 40 cigarettes per day were analyzed. To compare the lung cancer risks among male current smokers in Japan to those in the USA, adjusted rate ratios were obtained by Poisson regression analysis. The model included the natural logarithm of the number of lung cancer deaths as a response variable and the natural logarithm of person-years as an offset. Indicator variables for age group, number of cigarettes per day, and duration of smoking were used as covariates. Statistical computations were carried out using the SAS statistical package (version 8.02; SAS Institute, Cary, NC, USA).

Results

Current and former smokers in the Three-Prefecture Cohort Study showed a significantly increased risk of lung cancer death for both men and women compared with non-smokers (Table 1). A statistically significant dose–response trend of RR was observed for men and women current smokers (Table 2).

In the first 6 years of follow up, the Three-Prefecture Cohort Study had 341 deaths due to lung cancer (260 men and 81 women). Adjusted RR for current smokers versus non-smokers were 3.16 (95%CI 1.29–3.64) for men and 2.68 (95%CI 1.58–4.53) for women. Corresponding reported RR in the CPS-II study were 23.2 (95%CI 19.3–27.9) for men and 12.8 (95%CI 11.3–14.7) for women.

Death rates among current smokers and non-smokers were calculated, based on attained age (Fig. 1). Compared with the CPS-II, death rates among Japanese current smokers were lower in all age groups, with the exception of the youngest and oldest female age groups. In contrast, death rates among Japanese non-smokers were higher than those in the USA, for both men and women regardless of age. Cumulative death rates between 40 and 84 years and rate ratios are presented in Table 3. Compared with US non-smokers, Japanese non-smokers had a higher cumulative mortality of lung cancer with an approximately threefold increased risk for men and a twofold increased risk for women. However, Japanese current smokers were at a significantly 60% lower risk of lung cancer compared to those in the USA.

The mean number of cigarettes smoked per day (Fig. 2a) decreased with age for men and women in both Japan and the USA. However, current smokers in Japan had a lower daily