[CANCER RESEARCH 59, 1481–1484, April 1, 1999]

Increased Chromosome-Type Chromosome Aberration Frequencies as Biomarkers of Cancer Risk in a Blackfoot Endemic Area[1]

Saou-Hsing Liou, Jia-Chyi Lung, Yeong-Hwang Chen, Tsann Yang, Ling-Ling Hsieh,[2] Chien-Jen Chen, and Trong-Neng Wu

Department of Public Health, National Defense Medical Center [S-H. L., J-C. L., Y-H. C., T. Y.], Institute of Epidemiology, School of Public Health, National Taiwan University [C-J. C.], and Disease Surveillance and Quarantine Service, Department of Health [T-N. W.], Taipei 100; and Department of Public Health, ChangGung University, TaoYuan, 333 [L-L. H.], Taiwan, Republic of China

ABSTRACT

To examine whether biomarkers such as sister chromatid exchanges (SCEs) and chromosome aberrations (CAs) can predict cancer development, a nested case-control study was performed in a blackfoot endemic area with a known high cancer risk. A cohort of 686 residents was recruited from three villages in the blackfoot endemic area. Personal characteristics were collected, and venous blood was drawn for lymphocyte culture and stored in a refrigerator. The vital status and cancer development were followed using the National Death Registry, Cancer Registry, and Blackfoot Disease Registry. The follow-up period was from August 1991 to July 1995. During this 4-year period, 31 residents developed various types of cancer. Blood culture samples from nine of these subjects were unsuitable for experiments due to improper storage. Finally, a total of 22 cancer cases had cytogenetic samples that could be analyzed. Twenty-two control subjects were selected from those who did not develop cancer in the study period, and these subjects were matched to cases by sex, age, smoking habits, and residential area. The results showed that there was no significant difference in the frequencies of SCE and chromatid-type CAs between the case and control groups. However, the frequencies of chromosome-type CAs, *e.g.*, chromosome-type gaps, chromosome-type breaks, chromosome-type breaks plus exchanges, total chromosome-type aberrations, and total frequencies of CAs in the case group, were significantly higher than those in the control group ($P < 0.05$). The odds ratio of cancer risk in subjects with more than zero chromosome-type breaks was 5.0 (95% confidence interval = 1.09–22.82) compared to those with zero chromosomal breaks. The odds ratios for more than zero chromosome-type breaks plus exchanges and a frequency of total chromosome-type aberrations of >1.007% were 11.0 and 12.0, respectively ($P < 0.05$). Subjects with a total CA frequency of >4.023% had a 9-fold increase for cancer risk. These results indicate that chromosome-type CAs are good biomarkers for the prediction of cancer development, whereas SCEs and chromatid-type CAs cannot predict cancer risk.

INTRODUCTION

Biomarkers can provide useful information for early detection of the effects of carcinogen exposure and the prediction of cancer risk. Cytogenetic markers such as CAs[3] and SCEs have been considered to be markers of early biological effects of carcinogen exposure (1). The association between carcinogen exposure and increased frequencies of cytogenetic markers is well known and has been reported in several human epidemiological studies and animal studies (2–8). However, whether these cytogenetic markers are associated with the future development of cancer is still unclear. Two previous studies, one in Nordic countries and one in Italy, revealed that CA frequencies were associated with cancer development, whereas the frequencies of SCEs and MN were not associated with cancer risk (9–11).

Blackfoot disease is an endemic disease in the southern Taiwan, probably due to the intake of high concentrations of arsenic from the artesian well water. The incidence of cardiovascular disease and also various other types of cancers is significantly higher in the blackfoot endemic area than in other areas of Taiwan (12–16). We performed a nested case-control study in this high cancer risk area to evaluate the association between cytogenetic markers and cancer risk prediction. The objective of this study was to determine whether biomarkers, *e.g.*, SCE and CAs, can predict cancer development.

MATERIALS AND METHODS

Study Population. The arsenic-exposed study population was selected from residents of Pu-Dai county, Chiagi, Taiwan, which has the highest incidence of both blackfoot disease and cancer in the blackfoot endemic area. A nested case-control study design was used in this cohort study. A cohort of 686 residents with no known history of cancer was recruited in August 1991 from three villages, How-Mei, Fu-Hsing, and Hsing-Ming, which have the highest cancer risk in the blackfoot endemic area. The residents were examined by a physician and a dermatologist to check for possible preexisting cancers at the time of enrollment.

Data Collection. Demographic characteristics were collected by interviews with a structured questionnaire to evaluate the potential confounding influences in the data analysis. Data were collected by the questionnaire on variables including personal habits, such as smoking status and intake of alcohol and Chinese tea; residential and artesian water drinking history; occupational history; and disease or cancer history. Five ml of venous blood were collected in a heparinized vacutainer during the physical examination in August 1991 (17).

Cancer Ascertainment. Data on the vital status and cancer development was followed up for each subject in the cohort through the National Death Registry, National Cancer Registry, and the Blackfoot Disease Registry. Periodic follow-up once a year was also performed by the Blackfoot Disease Study Group of National Taiwan University. The follow-up period was from August 1991 to July 1995. To determine temporal relationships between cytogenetic markers and cancer development, cancer cases that developed in the first year of follow-up were excluded from this study. During the 4-year follow-up period, 31 residents developed cancer. However, nine of these cases had to be excluded due to dryness of the cell pellets. The remaining 22 cancer cases were defined as the case group.

Selection of the Control Group. Twenty-two controls were selected from the remainder of the cohort who did not develop cancer during the 4-year follow-up period. The controls were matched to cases by sex, age, smoking habits, and residential village.

Measurement of CAs and SCEs. After collection of blood specimens from the study population, the lymphocytes were cultured within 12 h of blood drawing. The same batch of medium and chemical solutions were used in all experiments. Whole blood (0.5 ml) was cultured at 37°C in 5 ml of RPMI 1640 supplemented with 15% FCS, 1% penicillin-streptomycin, and 1% L-glutamine for 72 h. Each culture was run in duplicate to ensure a sufficient number of mitoses for analysis. Two h before harvest, colchicine (15 μg/ml) was added to block the cells in metaphase. The cell pellets were then treated with 0.075 M KCl hypotonic solution and fixed with glacial acetic acid:methanol (1:3)

Received 8/17/98; accepted 1/29/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This study was supported by Grant DOH85-HR-516 and DOH86-HR-516 from the National Health Research Institute, Department of Health, The Executive Yuan, Republic of China.

[2] To whom requests for reprints should be addressed, at Department of Public Health, ChangGung University, 259 Wen-Hwa 1 Road, Kwei-San, TaoYuan, 333, Taiwan, Republic of China. Phone: 886-3-3288038; Fax: 886-3-3288038.

[3] The abbreviations used are: CA, chromosome aberration; SCE, sister chromatid exchange; MN, micronucleus; CI, confidence interval.

Table 1 *Sources and date of confirmation of cancer cases during the follow-up period from August 1991 to June 1995*

Sources	August 1991 to July 1992	July 1992 to December 1992	January 1993 to December 1993	January 1994 to June 1995	Total
Cancer registry	0	6	10	9	25
Death records	0	0	1	0	1
Telephone interview	0	0	3	0	3
Blackfoot disease registry	0	0	1	1	2
Total		6	15	10	31

Table 2 *Distribution of cancer types and residential areas for 31 cancer cases that developed during the follow-up period*

Cancer type	No.	Incidence (10^{-3}, $year^{-1}$)	Residential area: How-Mei (n = 122)	Fu-Hsing (n = 377)	Hsing-Ming (n = 187)
Lung	3	1.46	2	1	0
Thymus	1	0.49	0	1	0
Bladder	4	1.94	0	3	1
Colon	2	0.97	0	1	1
Vagina	1	0.49	0	0	1
Kidney	2	0.97	1	1	0
Stomach	1	0.49	0	1	0
Uterus	3	1.46	1	1	1
Skin	11	5.34	5	5	1
Pancreas	1	0.49	0	0	1
Liver	1	0.49	0	1	0
Prostate	1	0.49	0	0	1
Total	31	15.06	9	15	7

solution and stored in a mixture of fixative at 4°C in a refrigerator until scoring (17).

The chromosomes for scoring of CAs and SCEs were stained by the fluorescence plus Giemsa method as described previously (6, 17). All slides were blindly coded and scored by one investigator to minimize observer bias. The first-division metaphase with >42 chromosomes and good staining was selected for scoring of CAs. The second-division metaphase with >42 chromosomes and good differential staining was selected for the scoring of SCEs. One hundred first-division metaphases were randomly selected and scored as chromatid-type and chromosome-type CAs, including gaps, breaks, and exchanges. Twenty-five second-division metaphases were randomly selected and scored as SCEs/cell.

Statistical Methods. The distributions of characteristics in the cancer case and control groups were expressed as percentages for categorical variables and means for continuous variables. The frequencies of CAs (CAs/100 cells) and SCEs (SCEs/cell) were expressed as means and SDs. Nonparametric Mann-Whitney U test was used for comparing the differences in the frequencies of CAs and SCEs between groups. The association between CAs and cancer risk was estimated by odds ratio and 95% CI. The frequency of each type of CA was dichotomized into high- and low-frequency groups based on the median CA frequency of the control group. The odds ratio for cancer risk was calculated by comparing the proportion of cancer cases with a CA frequency higher than the median value to the proportion of cancer cases with a CA frequency lower than the median value.

RESULTS

Cancer Development during the Follow-Up Period. There were 31 cases of cancer that developed during the 4-year follow-up period (Table 1). Cancer cases diagnosed within 1 year of the beginning of the follow-up were excluded. Most of the cancer cases were confirmed in the cancer registry. However, five of these cases were identified only from telephone interviews and from blackfoot disease registry records. Most of the cancers developed during the year of 1993.

The dominant cancer type in this blackfoot endemic area was skin cancer (11 cases), followed by bladder cancer (4 cases), lung cancer (3 cases), and cancer of uterus and cervix (3 cases; Table 2). The incidence of cancer was estimated to be 15 per 1000 per year.

Distribution of Characteristics in Cancer and Control Groups. Because the cell pellets for cytogenetic counting of 9 cancer cases were dried during the storage, only the remaining 22 cancer cases and 22 matched controls were included in the data analysis. The distribution of age and personal habits is shown in Table 3. There was no significant difference between the cancer and control groups in the mean age and the mean duration of drinking artesian water. There was also no difference in the percentage of smoking, alcohol drinking, and tea consumption between these groups. Most of them were engaged in seafood harvesting. None of them had a history of exposure to other known mutagens or carcinogens. Adjustment for these variables will not be shown in the later because these variables are not significantly different between the case and control groups and they are not confounders.

Association between SCEs and Risk of Cancer. The mean SCE frequency in the 22 cancer cases was 6.73 ± 1.53 SCEs/cell, whereas it was 6.22 ± 1.11 SCEs/cell in the control group (Table 4). Although the frequency of SCE was higher in the cancer group than in the control group, this difference was not statistically significant.

To calculate the cancer risk of high SCE individuals, we dichotomized the distribution of SCE frequencies by the median of the control group (Table 4). The odds ratio of cancer risk in cases with an SCE frequency higher than 5.98 SCEs/cell was 1.25 (95% CI = 0.33–4.66). However, this increase in the odds ratio was not statistically significant.

Associations between Chromatid-Type CAs, Chromosome-Type CAs, and Risk of Cancer. The mean frequencies of chromosome-type and chromatid-type CAs in the cancer and control groups are shown in Table 5. The differences in CA frequencies between the cancer and control groups were statistically significant for all chromosome-type aberrations except for exchanges and for total aberrations. The mean frequencies of chromosome gap (1.4 ± 1.3), break (0.8 ± 1.0), and break plus exchange (1.2 ± 1.1) in the cancer group were significantly higher than in the control group (0.6 ± 0.8, 0.2 ± 0.4, and 0.3 ± 0.5, respectively). However, the frequency of exchange in the cancer group was not significantly higher than in the control group. The subtotal chromosome-type aberrations in the cancer group (2.6 ± 1.7) was also higher than in the control group (0.9 ± 1.0). The total number of aberrations, *i.e.*, the sum of chromosome-type and chromatid-type aberrations, was also significantly higher in the cancer group (6.1 ± 2.4) than in the control group (4.4 ± 2.6).

Table 3 *Distribution of age and personal characteristics among case and control groups[a] whose cytogenetic samples were included in the analysis*

Variables	Cases (n = 22)	Controls (n = 22)
Age (yr)	64.82 ± 7.85	63.91 ± 7.13
Smoker (%)	6 (27.3)	5 (22.7)
Duration of smoking (yr)	36.83 ± 13.01	46.20 ± 7.9
Quantity of smoking/day (cigarettes)	17.50 ± 7.58	17.00 ± 4.47
Alcohol drinking (%)	6 (27.3)	3 (13.6)
Tea drinking (%)	6 (27.3)	9 (40.9)
Duration of artesian water drinking (yr)	29.85 ± 11.90	32.63 ± 12.76

[a] No significant difference between these two groups.

Table 4 *Comparison of mean SCE frequencies between the case and control groups with or without dichotomization by median of the control group*

	Cases (n = 22)	Controls (n = 20)	P
SCE frequency (SCEs/cell)	6.73 ± 1.53	6.22 ± 1.11	0.36[a]
Dichotomized by median of control			
SCE frequency < 5.976	9 (40.9)[b]	10 (50.0)	
SCE frequency ≥ 5.976	13 (59.1)	10 (50.0)	0.88[c]

[a] Mann-Whitney U test.
[b] No. (%).
[c] χ^2 test; odds ratio = 1.25; 95% CI = 0.33–4.66.

Table 5 *Comparison of chromosome aberration frequencies (per 100 cells) between the case and control groups*

	Cases (n = 22)	Controls (n = 21)	P^a
Chromosome-type CAs			
Gap	1.4 ± 1.3[b]	0.6 ± 0.8	0.01
Break	0.8 ± 1.0	0.2 ± 0.4	0.01
Exchange	0.4 ± 0.7	0.1 ± 0.3	NS[c]
Break + exchange	1.2 ± 1.1	0.3 ± 0.5	0.001
Subtotal	2.6 ± 1.7	0.9 ± 1.0	<0.001
Chromatid-type CAs			
Gap	2.7 ± 1.2	2.5 ± 1.6	NS
Break	0.6 ± 1.0	0.9 ± 0.9	NS
Exchange	0	0	NS
Break + exchange	0.6 ± 1.0	0.9 ± 0.9	NS
Subtotal	3.3 ± 1.8	3.4 ± 2.0	NS
Tricentrics	0.1 ± 0.3	0	NS
Total CAs	6.1 ± 2.4	4.4 ± 2.6	0.018
Total breaks + exchanges	1.8 ± 1.4	1.2 ± 1.1	NS

[a] Mann-Whitney U test.
[b] Mean ± SD.
[c] NS, not significant.

The association of cancer risk with a high frequency of CAs was estimated by odds ratio by comparison of the number of cases with a higher CA frequency than median to the number of cases with a lower CA frequency than median for each type of aberration. The cutoff point value for each CA was set at the median CA frequency of the control group (Table 6). The odds ratio for cancer risk in subjects with more than zero chromosome-type breaks was 5.0 (95% CI = 1.09–22.82), compared to those with zero chromosomal breaks. The odds ratios for cancer risk for subjects with more than zero chromosome-type breaks plus exchanges and for subjects with a frequency of total chromosome-type aberrations of >1.007% were 11.0 (95% CI = 1.42–85.2) and 12.0 (95% CI = 1.56–92.3), respectively. The odds ratio for cancer risk for subjects with total CAs greater than 4.023% was 9.0 (95% CI = 1.14–71.04). No significant association was found between cancer risk and chromosome-type gaps, chromosome-type exchanges, and all chromatid-type aberrations.

DISCUSSION

Biomarkers of response indicate biological or biochemical changes in target tissue or in an established surrogate resulting from chemical actions. The prominent markers commonly used are cytogenetic changes, such as SCEs, CAs, and MN. Although the presence of cytogenetic markers themselves does not necessarily lead to adverse health outcomes, high levels of these markers apparently indicates that cells have been exposed to mutagens/carcinogens. Many studies support the association between cytogenetic changes (including SCEs, CAs, and MN) induced by exposure to carcinogens and the development of certain malignant and premalignant changes (1, 18). Such mutation events cause alterations in the activity or expression of growth control genes, which are the key steps in carcinogenesis (19–21). However, a clear connection between the presence of cytogenetic changes and cancer has not yet been established.

Here, we found that SCEs and chromatid-type CAs were not associated with cancer development in the 4-year follow-up period, whereas chromosome-type CAs were associated with cancer development in the follow-up period. These findings suggest that chromosome-type CAs are better biomarkers of future cancer risk than SCEs and chromatid-type CAs. These findings were compatible with two previous studies (9–11) in Nordic countries and in Italy, which found that CAs were associated with cancer development, whereas SCEs and MN were not. In a study in four Nordic countries, Hagmar *et al.* (9) found that an elevated cancer risk was associated with the highest tertile of CA frequency for all cancer sites combined. In Italy, Bonassi *et al.* (10) found a significantly elevated risk of mortality for all cancer types combined, which was more specific for respiratory tract cancer and lymphatic or hematopoietic cancer, was associated with the highest tertile of CA frequency.

There are methodological discrepancies between these studies and the present study. In our study, a high-risk population was selected and followed-up using a nested case-control design, and the measurements of cytogenetic markers was always performed in the same laboratory. However, in the Nordic and Italy study, cytogenetic data from several countries/laboratories were combined, and the cutoff point of cytogenetic markers was arbitrary due to large variations in measurements. Although there were important discrepancies in the methodology and study design of these investigations, their similar findings support the usefulness of CA in the prediction of cancer risk. These results suggest that CA may also be useful as an outcome measure in molecular epidemiological study.

The main rationale for using cytogenetic assays for biological monitoring is that genetic damage in a nontarget tissue, most often in the peripheral blood lymphocytes, reflects the occurrence of similar events in the cells of target tissues involved in carcinogenic processes. Thus, cytogenetic monitoring in peripheral lymphocytes may serve as an early indicator of DNA lesions. The results of this study provide support for the hypothesis that CAs measured in the peripheral lymphocytes reflect relevant events in the processes of carcinogenesis and may serve as surrogate end points for cancer risk. Detection of elevated frequencies of CAs enables assessment of the mutagenic potential of chemical exposure and prevention of severe effects due to overexposure (7, 8). In addition, CA is nonspecific, in the sense that it cannot discriminate between the effects of several different exposures. However, the frequency of CAs provides information about the

Table 6 *Odds ratio of cancer risk after dichotomization of CAs into groups higher and lower than the median frequency of controls*

	Cases (n = 22)	Controls (n = 21)	Odds ratio	95% CI
Chromosome-type CAs				
Gap				
0	4 (18.2)	11 (52.4)	1.00	
>0[a]	18 (81.8)	10 (47.6)	4.50	0.97–20.82
Break				
0	10 (45.5)	17 (81.0)	1.00	1.09–22.82[b]
>0	12 (54.5)	4 (19.0)	5.00	
Exchange				
0	16 (72.7)	19 (90.5)	1.00	0.58–42.80
>0	6 (27.3)	2 (9.5)	5.00	
Break + exchange				
0	6 (27.3)	15 (71.4)	1.00	1.42–85.20[b]
>0	16 (72.7)	6 (28.6)	11.00	
Total chromosome-type				
≤1.007	5 (22.7)	15 (71.4)	1.00	1.56–92.29[b]
>1.007	17 (77.3)	6 (28.6)	12.00	
Chromatid-type CAs				
Gap				
≤2.003	5 (22.7)	10 (47.6)	1.00	0.85–18.84
>2.003	17 (77.3)	11 (52.4)	4.00	
Break				
≤1.003	15 (68.2)	10 (47.6)	1.00	0.19–1.66
>1.003	7 (31.8)	11 (52.4)	0.56	
Break + exchange				
≤1.003	15 (68.2)	10 (47.6)	1.00	0.19–1.66
>1.003	7 (31.8)	11 (52.4)	0.56	
Total chromatid-type				
≤3.009	10 (40.5)	9 (42.9)	1.00	0.29–36.45
>3.009	12 (54.5)	12 (57.1)	1.00	
Total CAs				
≤4.023	5 (22.7)	12 (57.1)	1.00	1.14–71.04[b]
>4.023	17 (77.3)	9 (42.9)	9.00	
Total breaks + exchanges				
≤1.003	6 (27.3)	9 (42.9)	1.00	0.58–42.80
>1.003	16 (72.7)	12 (57.1)	5.00	

[a] Median CA frequency of control group.
[b] $P < 0.05$.

cumulative effects of combined exposure to carcinogens, which is highly associated with increased risk of cancer.

The results of this study indicate an association between CAs and cancer risk; however, it is still unclear whether this association is valid for other populations or for specific cancer types. Further research that extends the sample size of the study cohort is needed. In addition, obtaining more cancer cases will allow for analysis of the data stratified by cancer type, which will provide more specific information about the association between CA and cancer risk.

REFERENCES

1. Carrano, A. V. Chromosomal alterations as a marker of exposure and effect. J. Occup. Med., *28:* 1112–1116, 1986.
2. Wen, W. N., Lieu, T. L., Chang, H. J., Wu, S. W., Yau, M. L., and Jan, K. Y. Baseline and sodium arsenite-induced sister chromatid exchanges in cultured lymphocytes from patients with blackfoot disease and healthy persons. Hum. Genet., *59:* 201–203, 1981.
3. Ostrosky-Wegman, P., Gonsebatt, M. E., Montero, R., Vega, L., Barba, H., Espinosa, J., Palao, A., Cortinar, C., Garcia-Vargas, G., del Rozo, L. M., and Cebrian, M. Lymphocyte proliferation kinetics and genotoxic findings in a pilot study on individuals chronically exposed to arsenic in Mexico. Mutat. Res., *250:* 477–482, 1991.
4. Lerda, D. Sister-chromatid exchange (SCE) among individuals chronically exposed to arsenic in drinking water. Mutat. Res., *312:* 111–120, 1994.
5. Nordenson, I., Beckman, G., Beckman, L., and Nordstrom, S. Occupational and environmental risks in and around a smelter in northern Sweden. II. Chromosomal aberrations in workers exposed to arsenic. Hereditas, *88:* 47–50, 1978.
6. Liou, S. H., Jacobson-Kram, D., Poirier, M. C., Nguyen, D., Strickland, P. T., and Tockman, M. S. Biological monitoring of fire fighters: sister chromatid exchange and polycyclic aromatic hydrocarbon-DNA adducts in peripheral blood cells. Cancer Res., *49:* 4929–4935, 1989.
7. De Klein, A. Oncogen activation by chromosomal rearrangement in chronic myelocytic leukemia. Mutat. Res., *86:* 162–172, 1987.
8. Heim, S., and Mitelman, F. Nineteen of 26 cellular oncogenes precisely localized in the human genome map to one of the 83 bands involved in primary cancer specific rearrangements. Hum. Genet., *75:* 70–72, 1987.
9. Hagmar, L., Brogger, A., Hansteen, I. L., Heim, S., Hogstedt, B., Knudsen, L., Lambert, B., Linnainmaa, K., Mitelman, F., Nordenson, I., Reuterwall, C., Salomaa, S., Skerfving, S., and Sorsa, M. Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic study group on the health risk of chromosome damage. Cancer Res., *54:* 2919–2922, 1994.
10. Bonassi, S., Abbondolo, A., Camurri, L., Dal Pra, L., De Ferrari, M., Degrassi, F., Forni, A., Lambert, L., Lando, C., Padovani, P., Sbrana, I., Vecchio, D., and Puntoni, R. Are chromosome aberrations in circulating lymphocytes predictive of a future cancer onset in humans? Preliminary results of an Italian cohort study. Cancer Genet. Cytogenet., *79:* 133–135, 1995.
11. Hagmar, L., Bonassi, S., Stromberg, U., Brogger, A., Knudsen, L. E., Norppa, H., Reuterwall, C., and the European Study Group on Cytogenetic Biomarkers and Health. Chromosomal aberrations in lymphocytes predict human cancer: a report from the European study group on cytogenetic biomarkers and health (ESCH). Cancer Res., *58:* 4117–4121, 1998.
12. Tseng, W. P., Chu, H. M., How, S. W., Fong, J. M., Lin, C. S., and Yeh, S. Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. J. Natl. Cancer Inst. (Bethesda), *40:* 453–463, 1968.
13. Chen, C. J., and Wang, C. J. Ecological correlation between arsenic level in well water and age-adjusted mortality from malignant neoplasms. Cancer Res., *50:* 5470–5474, 1990.
14. Chen, C. J., Chuang, Y. C., Lin, T. M., and Wu, H. Y. Malignant neoplasms among residents of a blackfoot diseases-endemic area in Taiwan: high-arsenic artesian well water and cancers. Cancer Res., *45:* 5895–5899, 1985.
15. Chen, C. J., Chuang, Y. C., You, S. L., Lin, T. M., and Wu, H. Y. A retrospective study on malignant neoplasms of bladder, lung and liver in blackfoot disease endemic area in Taiwan. Br. J. Cancer, *53:* 399–405, 1986.
16. Bates, M. N., Smith, A. H., and Hopenhayn-Rich, C. Arsenic ingestion and internal cancers: a review. Am. J. Epidemiol., *135:* 462–476, 1992.
17. Liou, S. H., Gu, T. L., and Chen, C. J. Hypersensitivity to mitomycin C-induced sister chromatid exchange as a biomarker of past exposure to arsenic. Cancer Epidemiol. Biomark. Prev., *5:* 103–107, 1996.
18. Hsu, Y. H., Li, S. Y., Chiou, H. Y., Yeh, P. M., Liou, J. C., Hsueh, Y. M., Chang, S. H., and Chen, C. J. Spontaneous and induced sister chromatid exchanges and delayed cell proliferation in peripheral lymphocytes of Bowen's disease patients and matched controls of arseniasis-hyperendemic villages in Taiwan. Mutat. Res., *386:* 241–251, 1997.
19. Hulka, B. S. Overview of biological marker. *In:* B. S. Hulka, T. C. Wilcosky, and J. D. Griffin (eds.), Biological Markers in Epidemiology, pp. 3–15, Oxford: Oxford University Press, 1993.
20. Lambert, B., Lindblad, A., Holmberg, K., and Francesconi, D. The use of sister chromatid exchange to monitor human population for exposure to toxicologically harmful agents. *In:* S. Wolff (ed.), Sister Chromatid Exchange, pp. 149–182. New York: Wiley, 1982.
21. Weinberg, R. A. The genetic origins of human cancer. Cancer (Phila.), *61:* 1963–1968, 1988.

Increased Chromosome-Type Chromosome Aberration Frequencies as Biomarkers of Cancer Risk in a Blackfoot Endemic Area

Saou-Hsing Liou, Jia-Chyi Lung, Yeong-Hwang Chen, et al.

Cancer Res 1999;59:1481-1484.