Hiroshima Journal of Medical Sciences
Vol.36, No.4, 387~393, December, 1987
HIJM 36-53

Immunohistochemical Analysis of Colorectal Cancer among Atomic Bomb Survivors in Hiroshima

Masami YAMAMOTO[1], Tetsuro YAMAMOTO[2], Jotaro HATA[2], Hitoshi NAKAGAWA[2], Hirofumi NAKATSUKA[3] and Eiichi TAHARA[2]

1) *Department of Pathology, Hiroshima University Hospital, 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan*
2) *Department of Pathology, Hiroshima University School of Medicine, Hiroshima 734, Japan*
3) *Department of Surgery, Hiroshima University School of Medicine, Hiroshima 734, Japan*

(Received September 22, 1987)

Key words: Colorectal cancer, Atomic bomb survivors, Immunohistochemistry

ABSTRACT

In order to elucidate the biological characteristics of colorectal cancer among atomic bomb survivors in Hiroshima, a total of 159 cases of colorectal cancers comprising 73 cases in exposed atomic bomb survivors and 86 cases in non-exposed individuals were examined histologically and immunohistochemically for various functioning proteins. No statistical differences could be demonstrated in the incidence of various marker expressions of colorectal cancers between the exposed group and control group. However, comparison by the site of colorectal cancer showed that sigmoid colon cancers in the exposed group or high dose group showed a significantly higher frequency of glycoproteins such as α_1-antichymotrypsin (ACT), secretory component (SC), α_1-antitrypsin (AAT), and human chorionic gonadotropin (HCG) when compared with the control group. These results correlated well with the epidemiological data that the radiation effect on the incidence of colorectal cancer in atomic bomb survivors was most remarkable in the sigmoid colon.

Epidemiologically, it has been well demonstrated that malignant tumors, especially leukemia[7], lung cancer[3], cancer of salivary gland[2], and breast cancer[16], develop as the late effects of atomic bomb radiation. Recently, it has been reported that the elevated incidence of multiple myeloma[6] and cancers of thyroid[4], stomach[11], and ovary[18] among atomic bomb survivors is related to radiation exposure dose, but there are only few reports on colorectal cancer among atomic bomb survivors. According to a recent report on the Life Span Study (LSS) sample of the Radiation Effects Research Foundation (RERF), colon cancer has also been observed to be a radiation effect but not rectal cancer[9]. Nakatsuka, one of the present authors, has confirmed these results and observed that radiation elevates the incidence of colon cancer, that is cancer on the right side of the colon and sigmoid colon, but not cancer of the transverse and descending colon[13].

On the other hand, some reports have been made on the histopathological studies on the tumors of various organs among atomic bomb survivors[17,18,20], but studies have not yet been conducted on the functional characteristics of cancer among atomic bomb survivors. In this study, colorectal cancers in atomic bomb survivors and non-exposed cases in Hiroshima were examined histologically and immunohistochemically for various functioning proteins in order to elucidate the biological characteristics of colorectal cancer among atomic bomb survivors.

MATERIALS AND METHODS

For the radiation exposed group, 73 cases of colorectal adenocarcinoma in Hiroshima atomic bomb survivors whose radiation exposure doses have been estimated were used in this study. These cases were surgically resected during the period from 1966 to 1980. Thirty-four of the cases were male and 39 were female, the mean age being 66.4 years. Five cases were located in caecum, 7 cases in ascending colon, 9 cases in transverse colon, 5 cases in descending colon, 20 cases in sigmoid colon and 27 cases in rectum. For the control group, 86 cases of colorectal adenocarcinoma in non-exposed patients were used. These cases were surgically resected during the period from 1976 to 1984. Fourty-seven cases were male and 39 were female, the mean age being 62.0 years. Among them, 10 cases were located in caecum, 10 cases in ascending colon, 16 cases in transverse colon, 5 cases in descending colon, 19 cases in sigmoid colon, and 29 cases in rectum.

The formalin-fixed and paraffin-embedded specimens were cut into 4μm sections and stained with hematoxylin and eosin, Grimelius technique for argyrophil reaction and Masson-Fontana technique for argentaffin reaction. The peroxidase-antiperoxidase method was applied for demonstrating lysozyme (LZ), human chorionic gonadotropin (HCG), secretory component (SC), α_1-antitrypsin (AAT), α_1-antichymotrypsin (ACT), gastrin, somatostatin, glicentin, pancreatic polypeptide (PP), peptide YY (PYY), CEA, and Ca19-9 immunoreactivity, as described in detail elsewhere[14]. For the detection of serotonin immunoreactive cells, the avidin-biotin complex method with Vectastain ABC kit (Vector Lab., USA) was used. Anti-serotonin monoclonal serum was obtained from Sera Lab., England and employed at a 1:600 dilution. Anti-glicentin serum (R 4804) which was purchased from Professor Yanaihara, Laboratory of Bioorganic Chemistry, Shizuoka College of Pharmacy was used after dilution at 1:600[21]. Antibody for PYY was supplied by Milab, Sweden. Other antisera were obtained from Dako, Denmark and diluted 1:200. Peroxidase was stained with 3,3'-diaminobenzidine tetrahydrochloride containing 0.001% H_2O_2. Appropriate positive control slides were also stained as follows at the same time: gastric antral mucosa for AAT, ACT, gastrin, somatostatin and serotonin, pancreatic tissue for PP and glicentin, duodenal mucosa for LZ and SC, rectal mucosa for PYY, and choriocarcinoma for HCG. For the negative controls, non-immune rabbit or mouse serum were utilized in place of primary antibodies.

RESULTS

Histological classification of colorectal adenocarcinomas among the control group and exposed group is shown in Table 1. No difference could be observed between the two groups.

The incidence of endocrine cells and immunoreactivity of various tumor markers in the tumor tissues among the control group and exposed group is shown in Table 2. Although no statistical significant differences could be observed, the incidence of serotonin-immunoreactive cells and lysozyme-, AAT-, and SC-immunoreactive cells was higher in the exposed group than in the control group. Contrarily, the incidence of argyrophil cells in the control group was higher than in the exposed group, but no statistical difference could be observed. The incidence of various markers in the exposed group classified into three radiation dose groups is also shown in Table 2. Although there was no specific difference in the incidence of these markers among the radiation dose groups in the exposed group, in the high radiation dose group the incidence of serotonin- and

Table 1. Histological Type of Colorectal Cancer among Control Group and Exposed Group

Histologic type	Control Group	Exposed group
Well differentiated	47 cases (54.7%)	44 cases (60.3%)
Moderately differentiated	32 cases (37.2%)	22 cases (30.1%)
Poorly differentiated	5 cases (5.8%)	4 cases (5.5%)
Mucinous	2 cases (2.3%)	3 cases (4.1%)
Total	86 cases (100 %)	73 cases (100 %)

According to the classification of the Japanese Research Society for Cancer of Colon and Rectum.

Table 2. Incidence of Endocrine Cells and Immunoreactivity of Various Tumor Markers among Control Group and Exposed Group

Group	No. of cases with[a)]									
	Argyr	Argent	Serot	AAT	ACT	LZ	HCG	SC	CA19-9	CEA
Control (86 cases)	32 (37.2%)	5 (5.8%)	8 (9.3%)	5 (5.8%)	18 (20.9%)	12 (14.0%)	10 (11.6%)	38 (44.2%)	65 (75.6%)	84 (97.7%)
Exposed (73 cases)	21 (28.8%)	4 (5.5%)	11 (15.1%)	10 (13.7%)	14 (19.2%)	16 (21.9%)	10 (13.7%)	39 (53.4%)	49 (67.1%)	73 (100 %)
0 rad (27 cases)	8 (29.6%)	2 (7.4%)	4 (14.8%)	4 (14.8%)	4 (14.8%)	4 (14.8%)	5 (18.5%)	14 (51.9%)	16 (59.3%)	27 (100 %)
1 - 49 rad (35 cases)	11 (31.4%)	2 (5.7%)	5 (14.3%)	4 (11.4%)	7 (20.0%)	10 (28.6%)	3 (8.6%)	19 (54.3%)	26 (74.3%)	35 (100 %)
50+ rad (11 cases)	2 (18.2%)	0 (0 %)	2 (18.2%)	2 (18.2%)	3 (27.3%)	2 (18.2%)	2 (18.2%)	6 (54.5%)	7 (63.6%)	11 (100 %)

a) Argyr, argyrophil cell; Argent, argentaffin cell; Serot, serotonin-immunoreactive cell

Table 3. The Number of Synchronous Immunoreactivities for AAT, ACT, lysozyme, HCG, and SC in Control Group and Exposed Group

Group	No. of cases	No. of markers	Mean no. of markers
Control	86	83	1.0
Exposed	73	89	1.2
0 rad	27	31	1.1
1 - 49 rad	35	43	1.2
50+ rad	11	15	1.4

AAT-immunoreactive cells was slightly high and that of argyrophil cells was low. With regard to polypeptide hormone, three cases belonging to the exposed group showed glicentin-immunoreactivity, one of whom also showed PYY-immunoreactivity, but only one case of the control group contained PYY-, PP-, and somatostatin-immunoreactive cells. No case showed gastrin-immunoreactivity.

The synchronous expression of protease inhibitors and glycoproteins was examined. Table 3 shows the number of immunoreactivity for AAT, ACT, lysozyme, HCG, and SC synchronously expressed in the same tumor tissue of the control group and exposed groups. The mean number of markers in the exposed group, especially in high dose group, was higher than that in the control group. The number of cases expressing more than two markers was 21 in the exposed group and 22 in the control group. Among these cases, the expression of SC (16 cases), LZ (11 cases), ACT (10 cases), HCG (9 cases) and AAT (7 cases) was observed in the exposed group, whereas in the control group the expression of SC (18 cases), ACT (17 cases), LZ (11 cases), HCG (8 cases) and AAT (5 cases) was detected. Moreover, cases showing synchronous expressions of more than 4 markers were observed in 2 case of the control group and in 3 cases of the exposed group (Fig. 1).

Table 4 shows the incidence of various markers in tumor tissues by the location of cancers among the control group and exposed group. In sigmoid colon cancer, the incidence of ACT-, and SC-immunoreactivity was significantly higher in the exposed group than in the control group. Statistical difference could be observed in the incidence of SC-immunoreactivity between the two groups. In rectal cancer, the occurrence of argyrophil cells was lower with a significant difference in the exposed group than that in the control group.

As for sigmoid colon cancer, the incidence of markers among the control group and exposed group by radiation dose is shown in Table 5. Those exposed to a high radiation dose showed a significantly higher frequency of AAT-, HCG-, and SC-immunoreactivity than the control group.

DISCUSSION

Epidemiological studies made on the malignant tumors among atomic bomb survivors have been primarily focussed on the relationship between the risk of malignant tumors and radiation dose. There are, however, only a few reports on histopathologic studies of cancers among atomic bomb survivors. These reports could not demonstrate any evidence of difference in the histologic type of cancers in various organs, such

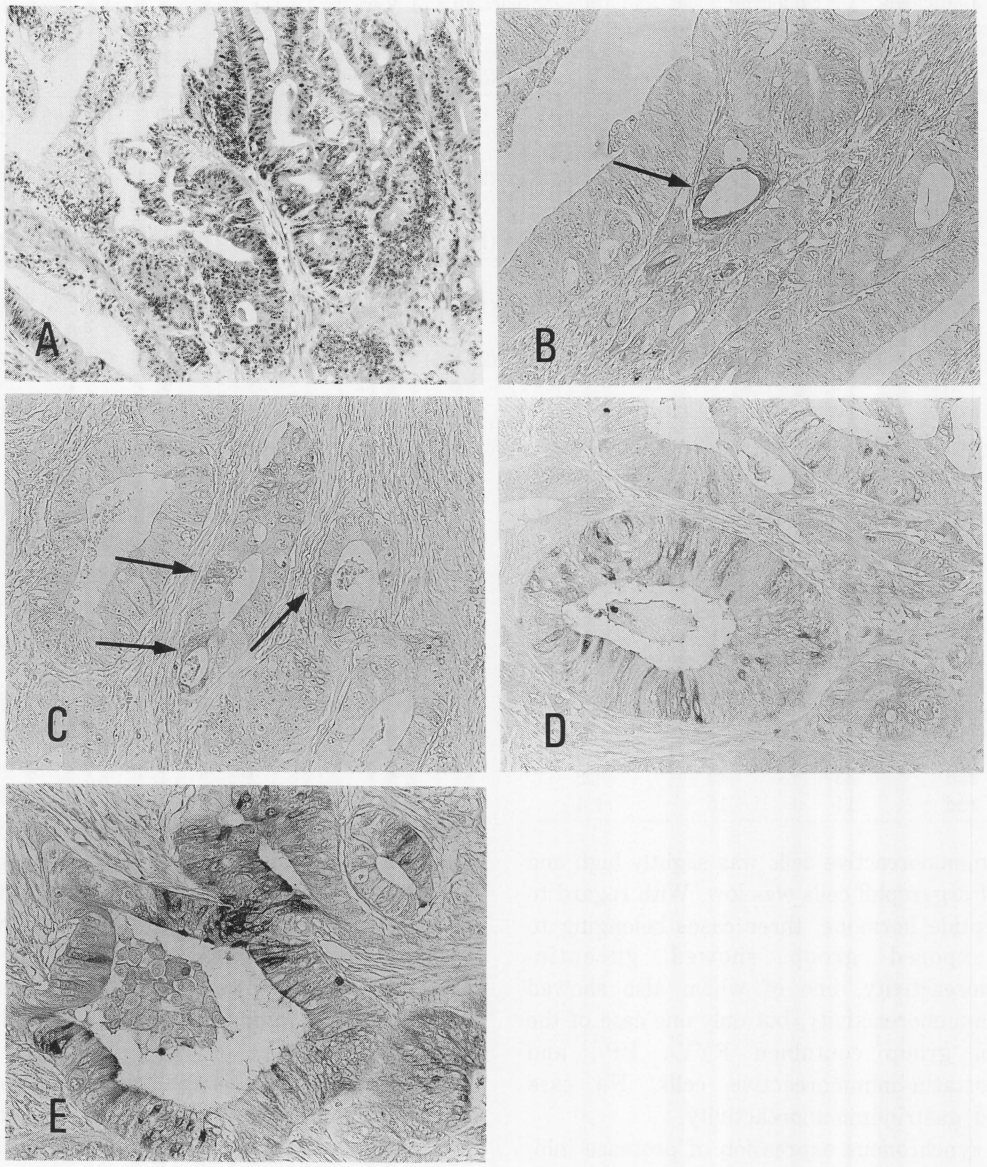

Fig. 1A-E. Photomicrographs of a case of well differentiated adenocarcinoma of sigmoid colon in atomic bomb survivor (radiation dose, 332 rad). Tumor cells show the immunoreactivity for HCG (B), ACT (C), AAT (D), and SC (E). Arrows: positive cells. A: HE stain, × 75 B-E: PAP method, × 150

as stomach[20], lung[20], breast[17], and ovary[18] between atomic bomb survivors and the non-exposed.

Nakatsuka et al recently observed that the risk of colon cancer among atomic bomb survivors increased with radiation dose and that the dose effect was especially pronounced for cancer of the sigmoid colon[13]. There was, however, no specific difference in histologic type of colon cancer between atomic bomb survivors and the non-exposed.

However, there has no report published on immunohistochemical analysis of the biological significance of cancers in atomic bomb survivors. This study was therefore made to elucidate the functional characteristics of colon cancer among atomic bomb survivors employing an immunohistochemical method for various functioning

Table 4. Incidence of Endocrine Cells and Immunoreactivity of Various Tumor Markers by Location of Colorectal Cancer among Control Group and Exposed Group

Location		No. of cases with									
		Argyr	Argent	Serot	AAT	ACT	LZ	HCG	SC	CA19-9	CEA
Caecum	Control (10 cases)	4	1	1	2	4	4	2	7	6	10
	Exposed (5 cases)	1	0	1	0	0	3	0	4	3	5
Ascending	Control (10 cases)	4	1	1	2	4	2	1	6	8	10
	Exposed (7 cases)	2	0	1	2	2	3	1	3	6	7
Transverse	Control (15 cases)	3	0	1	0	4	2	3	8	12	15
	Exposed (9 cases)	3	2	3	0	0	4	0	4	5	9
Descending	Control (5 cases)	3	1	2	1	1	2	1	4	4	5
	Exposed (5 cases)	3	0	2	3	2	1	2	2	5	5
Sigmoid	Control (19 cases)	3 (16%)	0 (0%)	0 (0%)	0 (0%)	2 (11%)	1 (5%)	1 (5%)	4 (21%)	16 (84%)	18 (95%)
	Exposed (20 cases)	7 (35%)	2 (10%)	3 (15%)	4 (20%)	8 (40%) b)	2 (10%)	4 (20%)	13 (65%) a)	13 (65%)	20 (100%)
Rectum	Control (27 cases)	15 (56%)	2 (7%)	3 (11%)	0 (0%)	3 (11%)	1 (4%)	2 (7%)	9 (33%)	19 (70%)	26 (96%)
	Exposed (27 cases)	5 (19%) a)	0 (0%)	1 (4%)	1 (4%)	2 (7%)	3 (11%)	3 (11%)	13 (48%)	17 (63%)	27 (100%)

a) $p<0.01$, χ^2 test; b) $0.05<p<0.1$

Table 5. Incidence of Endocrine Cells and Various Tumor Markers of Sigmoid Colon Cancer among Control Group and Exposed Group by Radiation Dose

Group	No. of cases with									
	Argyr	Argent	Serot	AAT	ACT	LZ	HCG	SC	CA19-9	CEA
Control (19 cases)	3 (16%)	0 (0%)	0 (0%)	0 (0%)	2 (11%)	1 (5%)	1 (5%)	4 (21%)	16 (84%)	18 (95%)
Exposed										
0 rad (10 cases)	4 (40%)	2 (20%)	1 (10%)	1 (10%)	3 (30%)	1 (10%)	2 (20%)	6 (60%)	7 (70%)	10 (100%)
1 - 49 rad (7 cases)	2 (29%)	0 (0%)	1 (14%)	0 (0%)	3 (43%)	0 (0%)	0 (0%)	4 (57%)	3 (43%)	7 (100%)
50+ rad (3 cases)	1 (33%)	0 (0%)	1 (33%)	3 (100%)	2 (67%)	1 (33%)	2 (67%)	3 (100%)	3 (100%)	3 (100%)

proteins.

In this study, the incidence of some markers such as AAT, SC, and lysozyme of the colorectal cancer was observed to be higher in the exposed group than in the control group, but the difference was not statistically significant. However, in comparing the site of colorectal cancer between the exposed group and control group, sigmoid colon cancers in the exposed group and in the high dose group in particular

showed a significantly higher frequency of glycoproteins such as ACT, SC, AAT, and HCG than the control group. This finding correlates well with the epidemiologic results that the radiation effect on the incidence of colorectal cancer was most remarkable in the sigmoid colon.

Among these markers, ACT, AAT and HCG have not been detected in the fetal colon[12,22]. ACT and AAT which have protease inhibitory activity are present in the small intestine and in the pyloric gland of the stomach[5,10]. Although there are a few reports on the distribution of these markers in carcinomas of the gastrointestinal tract, we and others have demonstrated these markers in gastric and gallbladder carcinoma[1,10,15,19]. As for colon cancer, Kittas et al reported that none of the 20 cases of colon cancer showed ACT- or AAT-immunoreactivity[10], but we have shown in this study with a large number of cases that colon cancer also contains ACT and AAT. HCG which has not found to be present in the gastrointestinal tract in the fetus and normal adult[12] has been observed in the gastrointestinal carcinomas[8,12]. Therefore, the expression of ACT, AAT and HCG in colon cancer might be an acquired phenomenon by the dysdifferentiation of cancer cells or might be derived from primitive undifferentiated cells which possess multi-differential capacity. At present, it is yet unknown what the higher incidence of these markers in the exposed group implies, but the result may indicate that colon cancer in atomic bomb survivors has a functionally multi-differential capacity and may have resulted from an abnormal cell differentiation induced by radiation.

On the other hand, rectal cancers in the exposed group showed a lower frequency of argyrophil cells than in the control group, although there was no correlation between the incidence of rectal cancer and radiation dose. The reason is yet unknown.

This study has shown a possibility that there are some biological characteristics in radiation induced cancers. Further studies with more markers such as oncogenes and growth factors must be made in order to elucidate the precise characteristics of colon cancers in atomic bomb survivors.

ACKNOWLEDGEMENTS

This study was supported in part by Grants in Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. **Aroni, K., Kittas, C., Papadimitriou, C.S. and Papacharalampous, N.X.** 1984. An immunohistochemical study of the distribution of lysozyme, a $_1$-antitrypsin and a_1-antichymotrypsin in the normal and pathological gall bladder. Virchows Arch. Pathol. Anat. **403**: 281—289.
2. **Belsky, J.L., Takeichi, N., Yamamoto, T., Cihak, R.W., Hirose, F., Ezaki, H., Inoue, S. and Blot, W.J.** 1975. Salivary gland neoplasms following atomic radiation: Additional cases and analysis of combined data in a fixed population, 1957—70. Cancer **35**: 555—559.
3. **Cihak, R.W., Ishimaru, T., Steer, A. and Yamada, A.** 1974. Lung cancer at autopsy in A-bomb survivors and controls, Hiroshima and Nagasaki, 1961—70: 1. Autopsy findings and relation to radiation. Cancer **33**: 1580—1588.
4. **Ezaki, H.** 1983. Thyroid carcinoma after exposure to atomic bomb radiation, Hiroshima 1958—79. J. Jpn. Pract. Surg. Soc. **44**: 1127—1137 (in Japanese).
5. **Geboes, K., Ray, M.B., Rutgeerts, P., Callea, F., Desmet, V.J. and Vantrappen, G.** 1982. Morphological identification of alpha-1-antitrypsin in the human small intestine. Histopathol. **6**: 55—60.
6. **Ichimaru, M., Ishimaru, T., Mikami, M. and Matsunaga, M.** 1982. Multiple myeloma among atomic bomb survivors in Hiroshima and Nagasaki, 1950—76: Relationship to radiation dose absorbed by marrow. J. Natl. Cancer Inst. **69**: 323—328.
7. **Ishimaru, T., Hoshino, T., Ichimaru, M., Okada, H., Tomiyasu, T., Tsuchimoto, T. and Yamamoto, T.** 1971. Leukemia in atomic bomb survivors, Hiroshima and Nagasaki, 1 October 1950—30 September 1966. Radiat. Res. **45**: 216—233.
8. **Ito, H. and Tahara, E.** 1983. Human chorionic gonadotropin in human gastric carcinoma. A retrospective immunohistochemical study. Acta Pathol. Jpn. **33**: 287—296.
9. **Kato, H. and Schull, W.J.** 1982. Studies of mortality of A-bomb survivors. 7. Mortality, 1950—78. Part 1. Cancer mortality. Radiat. Res. **90**: 395—432.
10. **Kittas, C., Aroni, K., Kotsis, L. and Papadimitriou, C.S.** 1982. Distribution of lysozyme, a_1-antichymotrypsin and a_1-antitrypsin in adenocarcinomas of the stomach and large intestine. An immunohistochemical study. Virchows Arch. Pathol. Anat. **398**: 139—147.
11. **Matsuura, H., Yamamoto, T., Sekine, I. and Otake, M.** 1984. Pathological and epidemiologi-

cal study of gastric cancer in atomic bomb survivors, Hiroshima and Nagasaki, 1959—77. Radiat. Res. **25**: 111—129.

12. **Miyayama, H. and Miyayama, Y.** 1985. Immunohistochemical study of αFP, CEA, hCG, and plac. Al-P expressed in human gastrointestinal cancers in comparison to embryo-fetal gastrointestinal mucosa. Pathol. Clin. Med. **3**: 905—915 (in Japanese).
13. **Nakatsuka, H.** 1985. Studies of colorectal cancer among atomic bomb survivors in Hiroshima, 1950—1980. Med. J. Hiroshima Univ. **33**: 147—163 (in Japanese).
14. **Tahara, E., Ito, H., Nakagami, K., Shimamoto, F., Yamamoto, M. and Sumii, K.** 1982. Scirrhous argyrophil cell carcinoma of the stomach with multiple production of polypeptide hormones, amine, CEA, lysozyme, and HCG. Cancer **49**: 1904—1915.
15. **Tahara, E., Ito, H., Taniyama, K., Yokozaki, H. and Hata, J.** 1984. $Alpha_1$-antitrypsin, $alpha_1$-antichymotrypsin, and $alpha_2$-macroglobulin in human gastric carcinomas: A retrospective immunohistochemical study. Human Pathol. **15**: 957—964.
16. **Tokunaga, M., Norman, J., Asano, M., Tokuoka, S., Ezaki, H., Nishimori, I. and Tsuji, Y.** 1979. Malignant breast tumors among atomic bomb survivors, Hiroshima and Nagasaki, 1950—74. J. Natl. Cancer Inst. **62**: 1347—1359.
17. **Tokunaga, M., Tokuoka, S. and Land, C.E.** 1986. Breast cancer in atomic bomb survivors. GANN Monograph on Cancer Research **32**: 167—177.
18. **Tokuoka, S.** 1986. Ovarian neoplasms in atomic bomb survivors. GANN Monograph on Cancer Research **32**: 179—189.
19. **Wittekind, Ch., Wachner, R., Henke, W. and von Kleist, S.** 1986. Localization of CEA, HCG, lysozyme, alpha-1-antitrypsin, and alpha-1-antichymotrypsin in gastric cancer and prognosis. Virchows Arch. Pathol. Anat. **409**: 715—724.
20. **Yamamoto, T., Nishimori, I., Tahara, E. and Sekine, I.** 1986. Malignant tumors in atomic bomb survivors with special reference to the pathology of stomach and lung cancer. GANN Monograph on Cancer Research **32**: 143—154.
21. **Yanaihara, N.** 1980. Immunochemical application of synthetic peptides to studies on the prophormone-hormone system. Biomed. Res. **1**: 105—116.
22. **Yokozaki, H.** 1986. Epithelial phenotypic expression of human foetal gastrointestinal mucosa: An immunohistochemical analysis. Hiroshima J. Med. Sci. **35**: 207—222.