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Progression of a Weakly Tumorigenic Mouse Fibrosarcoma at the Site of Early Phase of Inflammation Caused by Plastic Plates

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To elucidate tumor progression-enhancing factor(s), we examined the effects of host inflammation and host immunological status on *in vivo* tumor progression. One × 10^4 cells of QR clones (QR-32, -20 and -18), regressor tumor clones of 3-methylcholanthrene-induced fibrosarcoma, were unable to grow when injected s.c. into C57BL/6 mice in cell suspension form. However, QR clones grew and were lethal when s.c. implanted, attached to plastic plates. Furthermore, the tumor lines (QRpP) obtained from the tumors which had arisen from the plate-attached QR-32 clone cells no longer required plastic plates for their growth in normal mice, and had acquired stable malignant phenotypes. Although QR-32 cells became lethal when injected at the site of plastic plate implantation 1, 5 and 10 days before tumor injection, few tumors developed when plastic plates had been implanted 20 or 30 days before tumor injection. We established culture clones from the tumors arising in normal mice and mice immunosuppressed by irradiation. Clones derived from the tumors which had arisen in normal mice after implantation with plastic plates were lethal when re-implanted in normal mice (71%). On the other hand, clones derived from the tumors that arose in irradiated mice with or without plastic plates were lethal in only a few normal mice, when re-implanted (20 and 8%, respectively). These results indicate that QR clone cell progression is enhanced by the early phase of inflammation at the site of plastic plate implantation and that the progression-enhancing activity of co-implantation with a plastic plate is inhibited by previous whole-body irradiation of hosts.

Key words: Tumor progression — Inflammatory cell — Mouse fibrosarcoma — Plastic plate — Irradiation

Much evidence has indicated that tumors acquire various malignant phenotypes during *in vivo* growth.[1–5] It is crucial to identify the tumor progression-enhancing factors in order to prevent malignant progression of tumor cells. Augmentation of *in vivo* tumor-growth ability of tumor cells has been considered an important marker for tumor progression.[1,2,5] In general, when we examine alterations in tumor growth characteristics in syngeneic hosts, we often use tumor cells with basically tumorigenic properties and study the conversion of these cells to more malignant phenotypes. We, in our laboratory, have developed several spontaneously regressive experimental tumors which were isolated by persistent and nonlytic viral infection,[6–8] exposure to various types of chemicals,[9] *in vitro* cultivation[10,11] and appropriate gene transfections.[12,13] Since these regressor tumor cells are basically unable to grow progressively in syngeneic normal hosts, we can clearly observe their malignant progression as determined by the enhancement of *in vivo* growth ability. Therefore we have studied possible tumor progression-enhancing factors by using our regressor tumor cells.

Regressor QR clones[4] have been isolated from the high PGE_2-producing tumorigenic fibrosarcoma BMT-11 cl-9, originally induced by 3-methylcholanthrene in a C57BL/6 mouse.[9,14] QR clones and BMT-11 cl-9 cells are equally antigenic because CTL were induced to equal extents in mixed lymphocyte tumor culture, and they are equally sensitive to CTL-mediated cytotoxicity. Their *in vivo* tumorigenicity correlates well with the production of immunosuppressive PGE_2, which we have previously reported.[15] Restoration of *in vivo* growth of QR cells was observed when the QR cells were injected into immunosuppressed mice or were altered to produce large amounts of PGE_2.[1,15]

In this study, we used regressor QR clones and converted them to more malignant cells which acquired stable malignant phenotypes after implantation attached to a plastic plate. This system is a unique *in vivo* model for studying tumor progression. Therefore we used this experimental model to determine whether host inflammatory cells reactive to plastic plates are responsible for

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[4] Abbreviations: QR, a weakly tumorigenic and non-metastatic clonal cell line derived from BMT-11 cl-9; QR-32, a clonal cell line of QR clones; QRpP, a tumor cell line derived from the plate-attached QR-32 cells; PGE_2, prostaglandin E_2; BMT-11 cl-9, a C57BL/6 mouse fibrosarcoma; CTL, cytotoxic T lymphocytes.

the conversion of regressor cells to progressed ones or not. We also discuss the role of host immunological status in the enhancement of *in vivo* tumor progression.

MATERIALS AND METHODS

Animals Female C57BL/6 mice between 2 and 4 months of age were obtained from Clea Japan, Inc.

Tumor The origin and characteristics of the tumor cells used in this experiment have been described previously.[9, 14, 15] Briefly, we have isolated culture lines from the 3-methylcholanthrene-induced BMT-11 parental tumor in a C57BL/6 mouse and we obtained the clone BMT-11 cl-9. After exposure of the tumorigenic BMT-11 cl-9 cells to quercetin, followed by cell cloning, we obtained a number of clones which spontaneously regressed in normal syngeneic mice and were subsequently named QR clones.[9] QR clones exhibit spontaneous regression in mice after s.c. challenge with 2×10^5 cells, a dose that is four times greater than the minimum take dose of BMT-11 cl-9 cells, and they are therefore defined as weakly tumorigenic.[1, 9, 15] We have previously reported that the QR clone (QR-32) can be converted to tumorigenic phenotypes by host cell-derived factors which also induce QR cells to produce high amounts of immunosuppressive PGE_2.[1] In our previous studies,[15] we found that the threshold value of PGE_2-production required to suppress host immune reactivity *in vivo* was approximately 6,000 pg/ml, a value which *in vitro* is produced by 1×10^5 tumor cells during 24 h culture.

Culture conditions The QR clones (QR-18, -20 and -32) and QRpP tumor lines were maintained as a monolayer culture. All tumor cells were cultured in Eagle's minimum essential medium that contained 8% fetal calf serum (inactivated at 56°C for 30 min), sodium pyruvate, non-essential amino acids and L-glutamine at 37°C in a humidified 5% CO_2/95% air mixture. The conditions of the routine cell cultures of QR clones and QRpP tumor lines were previously reported.[1]

Cloning of tumor cells Cells were distributed into 96-well flat-bottomed microtiter plates (Nunc 1 67008) at an average concentration of 0.2 cells/well. Each clone was expanded *in vitro* and later injected s.c. into two syngeneic C57BL/6 mice at a dose of 2×10^5 cells per mouse.

Procedures for implantation of QR cells attached to plastic plates The procedures for the preparation and attachment of cells to plastic plates have been previously reported.[2] Briefly, the underside of culture dishes (polystyrene, Corning 25020) was cut into $1\times5\times10$ mm pieces (which we refer to as plastic plates) and sterilized by UV-irradiation. One$\times10^4$ QR-18, -20 or -32 cells in 50 μl of medium were seeded on each plastic plate. After 24 h incubation, plates with attached individual QR clones were separately implanted s.c. into normal syngeneic mice by insertion deep into a pocket under the skin, reaching up to the thorax, which was made with the tip of a sterilized scissors. The wounds were closed with sterile clips. In another experiment, QR-32 cells (1×10^5/0.1 ml) were injected into the area containing the pre-inserted plastic plate. Tumors developed at the site of plastic plate implantation and not at the wound site. In no case was a tumor observed at the wound sites. Average tumor diameters were measured twice weekly with vernier calipers.

Observation of *in vivo* growth of tumors In this study, we have used the weakly malignant QR clones, and defined tumor progression as the conversion of tumor cells to a more malignant phenotype that exhibits such properties as acquired tumorigenicity, invasive and metastasizing potential and, ultimately, the ability to kill the host more rapidly. Mouse survival was observed up to 100 days after tumor implantation.

Experimental pulmonary metastasis Normal C57BL/6 mice were intravenously injected with 1×10^6 cells in 0.2 ml of phosphate-buffered saline, and killed 19 days later. The lungs were fixed with Bouin's solution, and the macroscopic metastatic nodules on the lung surface were counted. Neither QR-32 clone nor QRpP-1–5 tumor lines produced any macroscopic metastatic nodules on the surface of the liver, spleen or kidney.

Preparation and radioimmunoassay for PGE_2 One$\times10^5$ QR-32 cells and QRpP-1–5 tumor cells were cultured in 24-well plastic plates in 2 ml of medium. The supernatants were stored at below −70°C until assay for PGE_2. PGE_2 was measured by using a commercially available radioimmunoassay kit (New England Nuclear, Boston, Mass.). The details of this assay have already been described elsewhere.[15]

^{60}Co irradiation C57BL/6 mice were immunosuppressed by 10 Gy whole-body ^{60}Co irradiation from a Toshiba ^{60}Co tele-therapy unit (RCR-120-C3) 6 h prior to tumor cell injection.

Statistical analysis The statistical significance of differences in tumor incidence was evaluated by χ^2 test and that in the case of PGE_2 production, by Student's t test.

RESULTS

Growth of QR clones implanted attached to plastic plates in syngeneic mice QR clone cells (QR-32, -20 and -18) exhibit spontaneous regression in normal mice after s.c. challenge with 1×10^4 cells in cell suspension form. Table I shows that QR-32, -18 and -20 cells which had been individually attached to plastic plates killed 14 out of 24 animals (58%, $P<0.001$), 5 out of 11 animals (45%, $P<0.05$) and 5 out of 12 animals (42%, $P<0.05$), respectively. The tumor growth in individual animals receiving QR-32 cells is summarized in Table I, and

specific growth curves are shown in Fig. 1A. Tumor growth was detected in six out of 10 animals at 32 days after co-implantation, and the tumors grew progressively. On the other hand, in four of the remaining animals, the tumor spontaneously regressed. QR-32 cells in cell suspension form produce no tumor (Fig. 1B). We obtained several tumor lines from the tumors which arose in mice after s.c. implantation of QR-32 cells attached to a plastic plate, and we named these lines QRpP-1, -2, -3, -4 and -5.

Tumorigenicity and PGE_2 production by QR-32 cells and QRpP tumor lines As shown in Table II, we next compared the biological characteristics of QRpP tumor lines with those of the original QR-32 cells. We observed that QRpP-1, -2, -4 and -5 tumor lines produced significantly higher amounts of PGE_2 than the QR-32 cells during *in vitro* cell culture and this corresponds well with the acquisition of *in vivo* tumorigenicity of the tumor lines even in the absence of a plastic plate. Although no spontaneous lung metastasis was observed when QR-32 cells were injected s.c. into normal mice, QRpP tumor lines showed augmented lung-colonizing ability after i.v. injection as compared with the parent QR-32 cells.

Table III shows that the biological characteristics of QRpP tumor lines such as s.c. tumorigenicity, as well as the *in vitro* PGE_2 production, remained stable for up to 6 months when cells were maintained under the routine culture conditions. No significant differences were observed in their growth rates and doubling times during long-term culture attached to a plastic plate (culture conditions *in vitro*). This suggests that the emergence of the QRpP tumor lines was due to *in vivo* growth at the site of plastic plate implantation rather than direct contact with the plastic plate.

Influence of timing of tumor injection after plastic plate implantation on QR-32 cell growth We next investigated the influence of timing of QR-32 cell injection at the site of plastic plate implantation upon the induction of progressive growth of QR-32 cells (Table IV). One$\times 10^5$ QR-32 cells were injected at the site where a plastic plate had been implanted 1, 5, 10, 20 or 30 days previously. We observed that QR-32 cells were able to grow progressively in 11 out of 13 (85%), 9 out of 14 (64%) and 9 out of 14 (64%) mice when QR-32 cells were injected

Table I. Growth of QR Clones Implanted Attached to Plastic Plates in Syngeneic C57BL/6 Mice

Clones	Tumorigenicity[a] died/used (%)	
	Plate (+)	Plate (−)
QR-32	14/24[b] (58)	0/12 (0)
QR-18	5/11[c] (45)	0/10 (0)
QR-20	5/12[c] (42)	0/12 (0)

a) One$\times 10^4$ QR clone cells were attached to a plastic plate ($10\times5\times1$ mm) and then s.c. implanted into the back of normal C57BL/6 mice.
b, c) *b*; $P<0.001$, *c*; $P<0.05$ versus animals injected with the original QR clone cells alone.

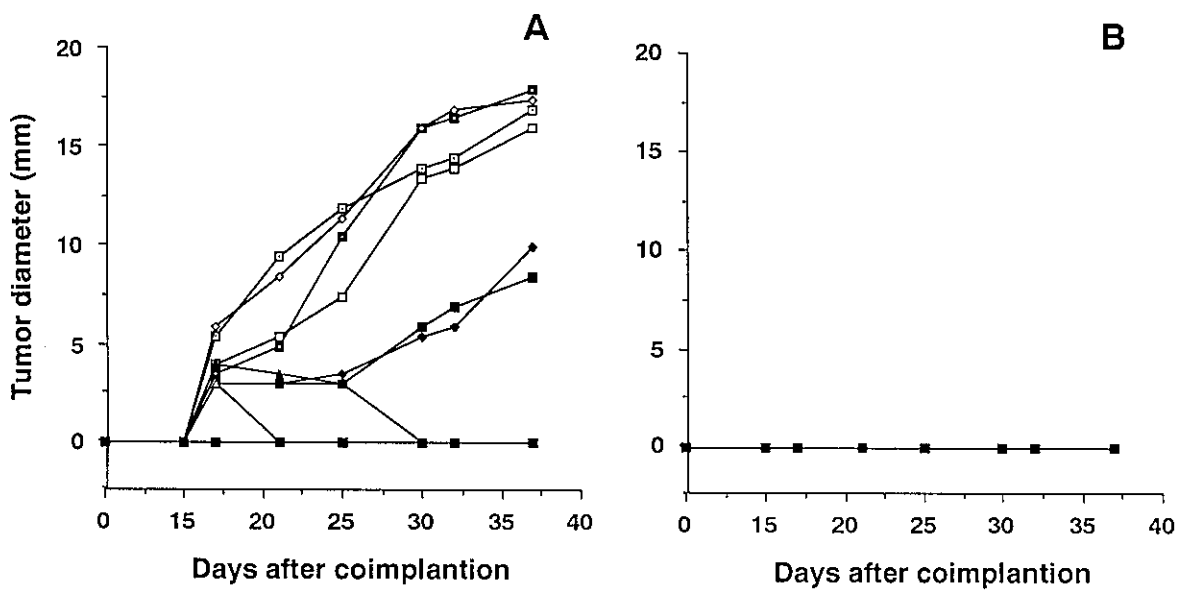

Fig. 1. Growth curves of QR-32 cells implanted attached to a plastic plate (A) or not (B) into normal syngeneic mice. Ten and 5 mice were used respectively.

Table II. Tumorigenicity and Prostaglandin E_2 Production by QR-32 Cells and QRpP Tumor Lines

Cells[a]	Tumorigenicity		PGE_2-production[g] (mean±SD, pg/ml)
	Subcutaneous injection[b] (died/used)	Intravenous injection[f] (No. of colonies per mouse lung)	
QR-32	0/8	0, 0, 0, 0, 0, 0, 0, 0	1,733±115
QRpP-1	8/8[c]	>150, >150, >150, >150, >150	7,833±289[h]
QRpP-2	6/8[d]	0, 3, 5, 6, 11, 13, 40	12,667±2,517[i]
QRpP-3	0/7[e]	3, 3, 4, 5, 5, 68	3,167±744[j]
QRpP-4	6/7[d]	6, 7, 11, >150, >150	All >25,000[h]
QRpP-5	3/8[e]	7, 8, 15, 80, 82	4,733±462[h]

a) One×10^4 QR-32 cells were attached to a plastic plate in culture and implanted s.c. into normal mice. The resultant tumors were cultured separately and named QRpP-1 to QRpP-5.
b) Normal mice were injected s.c. with 2×10^5 QRpP tumor cells or QR-32 cells.
c, d, e) *c*; $P<0.001$, *d*; $P<0.01$, *e*; not significant versus animals injected s.c. with QR-32 cells.
f) In a separate experiment, mice were i.v. injected with 1×10^6 of one of the six tumor cell types. Nineteen days later, the mice were killed and the metastatic nodules on the lung surface were counted microscopically. Each value represents the number of colonies per mouse lung.
g) PGE_2 levels in supernatants obtained from 1×10^5 tumor cell cultures in 24-well plastic plates in 2 ml of medium for 24 h. Determinations were carried out in triplicate and a mean and standard deviation were obtained.
h, i, j) *h*; $P<0.001$, *i*; $P<0.005$, *j*; not significant versus PGE_2 production by QR-32 cells.

Table III. Stability of the Biological Characteristics of QR-32 Cells and QRpP Tumor Lines during Long-term Culture

Cells	Cells maintained in culture for[a]:			
	0 months	1 month	3 months	6 months
In vivo tumorigenicity (died/used)[b]				
QR-32	0/8	0/5	0/5	0/5
QRpP-1	8/8	3/3	3/3	4/4
QRpP-2	6/8	5/5	5/5	3/3
QRpP-3	0/7	0/5	0/5	0/5
QRpP-4	6/7	3/3	5/5	3/3
In vitro PGE_2 production by tumor cells (pg/ml)[c]				
QR-32	1,600	1,400	1,600	1,800
QRpP-1	7,500	8,000	8,000	7,800
QRpP-2	13,000	13,000	10,500	11,000
QRpP-3	3,000	2,800	2,800	2,800
QRpP-4	>25,000	>25,000	>25,000	>25,000

a) QR-32 cells and QRpP tumor lines were maintained under routine culture conditions for the indicated periods.
b) Mice were injected s.c. with 2×10^5 of one or other type of tumor cell.
c) PGE_2 production during *in vitro* culture of QR-32 and QRpP tumor cells was measured by the same procedure as described in Table II, *g*.

at the site where a plastic plate had been implanted 1, 5 and 10 days previously, whereas tumors developed in only 1 out of 14 (7%) and 3 out of 13 (23%) mice when a plastic plate had been implanted 20 and 30 days before, respectively. These results indicate that host reactive cells involved in the early phase of inflammatory response to the plastic plate enhance *in vivo* growth of QR-32 cells in immunocompetent hosts.

QR-32 cell growth without acquired tumor progression in irradiated mice We next investigated the influence of irradiation of hosts on the *in vivo* growth and the progression of QR-32 cells. As shown in Table V Exp. A, in immunocompetent mice which were implanted with plastic plate-attached QR-32 cells, 6 out of 10 (60%) mice developed progressively growing tumors. When QR-32 cells were implanted in mice immunosuppressed by

irradiation, whether the cells were attached to a plastic plate or not, all the mice developed tumors.

We established culture clones from the tumors arising in normal and irradiated mice. The clones established from tumors developed in normal mice were re-injected s.c. (2×10^5 cells) into normal mice. Twelve out of 17 (71%) clones grew progressively in mice as indicated in Exp. B. On the other hand, clones obtained from the tumors arising in irradiated mice with or without a plastic plate in Exp. A grew progressively in only 3 out of 15 (20%) and in 1 out of 13 (8%) clones in normal mice, respectively in Exp. B. All of the 14 subclones of the cultured QR-32 cells spontaneously regressed when injected s.c. in normal mice.

PGE_2 production by the clones obtained from the resultant tumors in normal mice was significantly enhanced as compared with that of the 14 subclones of cultured QR-32 cells. PGE_2 production by the clones obtained from tumors in the immunosuppressed mice of Exp. A was not significantly enhanced as compared with that of the subclones of QR-32 cells.

Table IV. Influence of Tumor Cell Injection Timing after the Plastic Plate Implantation on QR-32 Cell Growth

Days after plastic plate implantation[a]	Tumorigenicity[b] (died/used)		
	Exp. 1	Exp. 2	Total (%)
Without plate	0/5	0/8	0/13 (0)
1	6/7	5/6	11/13[c] (85)
5	6/7	3/7	9/14[c] (64)
10	7/7	2/7	9/14[c] (64)
20	0/7	1/7	1/14[d] (7)
30	2/7	1/6	3/13[d] (23)

a) The number of days following plastic plate implantation into the back of normal mice.
b) One$\times10^5$ QR-32 cells were injected at the site where the plastic plate had been implanted.
c, d) *c*; $P<0.001$, *d*; not significant versus the animals injected s.c. with QR-32 cells without a plastic plate.

DISCUSSION

Regressor QR clones are converted to grow progressively in mice after implantation attached to plastic plates. These progressively growing tumors were also found to acquire stable malignant phenotypes, such as progressive growth in mice even in the absence of a plastic plate, the ability to form colonies in lungs after intravenous injection and the production of large amounts of immunosuppressive PGE_2 as compared with the original QR clone. Therefore, we define this phenomenon as *in vivo* tumor progression.

Tumor clones obtained from the tumors arising in normal mice which were implanted with QR-32 cells attached to plastic plates, exhibit progressive growth in normal mice in the absence of a plastic plate. In contrast, although QR-32 cells grow progressively in irradiated mice in the presence or absence of a plastic plate, only a few clones derived from the arising tumors were found to grow progressively in normal syngeneic mice. These observations reveal that tumors growing in irradiated mice

Table V. QR-32 Cell Growth without Acquired Tumor Progression in Irradiated Mice

Experiment A: Tumorigenicity of QR-32 cells in various conditions[a]			Experiment B: Characteristics of the clones derived from resultant tumors	
In	Attached to plastic plate	Tumor take/used mice	Tumorigenicity after s.c. injection in normal mice[b] Tumor take clone/used clones (%)	PGE_2 production in culture[c] (mean±SD, pg/ml)
Normal mice	Yes	6/10	12/17[f] (71)	5,580±1,930[f]
Irradiated mice[d]	Yes	8/8	3/15[g] (20)	1,645±1,740[g]
	No	5/5	1/13[g] (8)	2,880±1,593[g]
Culture[e]	No	0/8	0/14 (0)	2,170±1,436

a) One$\times10^4$ QR-32 cells attached to a plastic plate or suspended in PBS and implanted s.c. into the back of normal or irradiated mice.
b) The resultant tumors were cultured and clones were isolated by the limiting dilution procedure. Two$\times10^5$ cells of each clone were injected s.c. into normal mice.
c) PGE_2 production during *in vitro* culture of QR-32 and each of the tumor clone types was measured by the same procedure as described in Table II, *g*.
d) C57BL/6 mice were irradiated with 10 Gy, 6 h before injection with the tumor cells.
e) QR-32 cells were grown under culture conditions.
f, g) *f*; $P<0.001$, *g*; not significant versus tumor cells grown under culture conditions.

show only temporary growth advantages owing to the suppression of host immune responses, and have not acquired progressive growth characteristics. These findings suggest that the tumor progression is a result not of *in vivo* growth of tumor cells but of interaction of the tumor cells with host immunocompetent cells.

Results indicate that early-phase inflammatory cells, induced by plastic plate implantation, were responsible for the progression of QR-32 cells and that progression-enhancing activities of these reactive cells were inhibited by immunosuppressive irradiation. Studies concerning the role of foreign bodies in carcinogenesis and tumor progression have been reported.[16-19] Subcutaneous implantation of BALB/3T3 or C3H10T1/2 cells attached to a plastic plate in syngeneic normal mice produce hemangioendotheliomas. It was suggested that anchorage to plastic plates accelerated the process of carcinogenesis.[16-20] The precise mechanisms of neoplastic transformation of these normal cell lines after co-implantation with a plastic plate remain unclear. Recently, we have reported that rat mammary adenocarcinoma (ER-1 cells) with a low tumorigenic potential are converted to more malignant tumors by implantation attached to plastic plates.[2] When we compare the rat ER-1 cells with mouse QR-32 cells, two differences can be seen in their conversion to malignant tumors. First, they are sensitive or responsive to different stages of plate-reactive cells; i.e., to convert to a more malignant phenotype, rat ER-1 cells require late-stage plate-reactive cells (reactive cells which appear more than 30 days after plate implantation), whereas mouse QR-32 cells require early-stage plate-reactive cells (less than 20 days). Second, the mechanisms involved in the acceleration of tumor progression seem to be different. Rat ER-1 cells need growth factor(s), mainly produced by plate-reactive fibroblasts, and the activity of this soluble facter(s) would still be active in the plate-surrounding tissue-conditioned medium. In mouse QR-32 cells, as we have already reported,[1] the active radicals produced by foreign body-reactive cells may be involved in QR-32 cell progression. In fact, we could not succeed in inducing the progression of QR-32 cells after culturing in medium conditioned by plate-reactive cells (data not shown). The results of the previous and present studies in our laboratory suggest that there are diverse mechanisms responsible for foreign body-induced progression of tumor cells, and that the diversity may be due to the origin of the tumors.[1,2] Nevertheless, the inflammatory responses at the site of tumor growth appear to participate in tumor progression. Our earlier *in vitro* studies indicate that QR-32 cells produced large amounts of PGE_2 when cocultured with host effector cells.[1] In this assay, it is possible to detect progression-enhancing factor(s). Our preliminary data from this assay suggest that host effector cells such as lymphokine-activated killer cells, natural killer cells, polymorphonuclear leukocytes, and activated or resident macrophages are involved in the progression of the tumor cells (manuscript in preparation). Therefore we suspect that the early inflammatory cells should be one or more of those effector cell types. Those host effector cells are radiation-sensitive,[21] which supports our speculation. We are now trying to identify the types of early inflammatory cells at the histological level.

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REFERENCES

1) Okada, F., Hosokawa, M., Hamada, J-I., Hasegawa, J., Kato, M., Mizutani, M., Ren, J., Takeichi, N. and Kobayashi, H. Malignant progression of a mouse fibrosarcoma by host cells reactive to foreign body (gelatin sponge). *Br. J. Cancer*, **66**, 635–639 (1992).
2) Hamada, J-I., Takeichi, N., Okada, F., Ren, J., Li, X., Hosokawa, M. and Kobayashi, H. Progression of weakly malignant clone cells derived from rat mammary carcinoma by host cells reactive to plastic plate. *Jpn. J. Cancer Res.*, **83**, 483–490 (1992).
3) Fisher, E. R., Shoemaker, R. H. and Sabnis, A. Relationship of hyperplasia to cancer in 3-methylcholanthrene-induced mammary tumorigenesis. *Lab. Invest.*, **33**, 33–42 (1975).
4) Pitot, H. C. The natural history of neoplastic development: progression. *In* "Fundamentals of Oncology," ed. H. C. Pitot, pp. 163–200 (1986). Marcel Dekker, Inc., New York.
5) Pitot, H. C. Progression: the terminal stage in carcinogenesis. *Jpn. J. Cancer Res.*, **80**, 599–607 (1989).
6) Kobayashi, H., Sendo, F., Shirai, T., Kaji, H., Kodama, T. and Saito, H. Modification in growth of transplantable rat tumors exposed to Friend virus. *J. Natl. Cancer Inst.*, **32**, 413–419 (1969).
7) Kobayashi, H., Sendo, F., Kaji, H., Shirai, T., Saito, H., Takeichi, N., Hosokawa, M. and Kodama, T. Inhibition of transplanted rat tumors by immunization with identical tumor cells infected with Friend virus. *J. Natl. Cancer*

Inst., **44**, 11–19 (1970).
8) Kobayashi, H. Viral xenogenization of intact tumor cells. *Adv. Cancer Res.*, **30**, 279–299 (1979).
9) Ishikawa, M., Okada, F., Hamada, J-I., Hosokawa, M. and Kobayashi, H. Changes in the tumorigenic and metastatic properties of tumor cells treated with quercetin or 5-azacytidine. *Int. J. Cancer*, **39**, 338–342 (1987).
10) Yamashina, K., Oikawa, T., Kasai, M., Naiki, M., Chiba, I. and Kobayashi, H. Development of highly immunogenic variants of a rat fibrosarcoma line during *in vitro* cultivation. *Cancer Immunol. Immunother.*, **21**, 45–50 (1986).
11) Chiba, I., Oikawa, T., Naiki, M., Takimoto, M., Miyoshi, I., Mizuno, S., Yamashina, K., Yamagiwa, S. and Kobayashi, H. Enhanced immunogenicity of the cultured rat fibrosarcoma KMT-17 by cultivation in a low concentration of fetal calf serum. *Cancer Res.*, **47**, 1815–1819 (1987).
12) Itaya, T., Yamagiwa, S., Okada, F., Oikawa, T., Kuzumaki, N., Takeichi, N., Hosokawa, M. and Kobayashi, H. Xenogenization of a mouse lung carcinoma (3LL) by transfection with an allogeneic class I major histocompatibility complex gene (H-2L^d). *Cancer Res.*, **47**, 3136–3140 (1987).
13) Sugiura, C., Itaya, T., Kondoh, N., Oikawa, T., Kuzumaki, N., Takeichi, N., Hosokawa, M. and Kobayashi, H. Xenogenization of tumor cells by transfection with plasmid containing *env* gene of Friend leukemia virus. *Jpn. J. Cancer Res.*, **79**, 1259–1263 (1988).
14) Ishikawa, M., Hosokawa, M., Oh-hara, N., Niho, Y. and Kobayashi, H. Marked granulocytosis in C57BL/6 mice bearing a transplanted BMT-11 fibrosarcoma. *J. Natl. Cancer Inst.*, **78**, 567–571 (1987).
15) Okada, F., Hosokawa, M., Hasegawa, J., Ishikawa, M., Chiba, I., Nakamura, Y. and Kobayashi, H. Regression mechanisms of mouse fibrosarcoma cells after *in vitro* exposure to quercetin: diminution of tumorigenicity with a corresponding decrease in the production of prostaglandin E_2. *Cancer Immunol. Immunother.*, **31**, 358–364 (1990).
16) Boone, C. W. Malignant hemangioendotheliomas produced by subcutaneous inoculation of BALB/3T3 cells attached to glass beads. *Science*, **188**, 68–70 (1975).
17) Boone, C. W. and Jacob, J. B. Sarcomas routinely produced from putative nontumorigenic BALB/3T3 and C3H10T1/2 cells by subcutaneous inoculation attached to plastic plates. *J. Supramol. Struct.* **5**, 131–137 (1976).
18) Boone, C. W., Takeichi, N., Paranjpe, M. and Gilden, R. Vasoformative sarcomas arising from BALB/3T3 cells attached to solid substrates. *Cancer Res.*, **36**, 1626–1633 (1976).
19) Boone, C. W., Takeichi, N., Eaton, S. and Paranjpe, M. "Spontaneous" neoplastic transformation *in vitro*: a form of foreign body (smooth surface) tumorigenesis. *Science*, **204**, 177–179 (1979).
20) Rubin, H. Adaptive changes in spontaneously transformed Balb/3T3 cells during tumor formation and subsequent cultivation. *J. Natl. Cancer Inst.*, **72**, 375–381 (1984).
21) Bernardo, E. D. The effects of radiation on the cellular components of the immune system. *In* "Radiation Biology," ed. D. J. Pizzarello, pp. 193–204 (1982). CRC Press, Inc., Boca Ratton, Florida.