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Angiogenesis-Inducing Ability of Human Bladder Epithelium Cell Lines and "Spontaneously" Transformed Murine Fibroblasts (41752)

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Abstract. Ten human bladder epithelium cell lines were tested for their ability to induce blood vessel formation after intradermal injection into irradiated ST/a mice. Cell lines that were shown to be tumorigenic in nude mice, were able to evoke angiogenesis of a higher intensity than nontumorigenic cell lines. No difference was observed between the angiogeneic ability of tumorigenic cells originating from tumors and from *in vitro* transformed urothelium of nontumor origin. Similarly the origin of nontumorigenic urothelial cell lines did not show any influence on their angiogeneic abilities, but nontumorigenic cell lines which had undergone "infinite growth transformation" exhibited a higher angiogeneic activity than nontumorigenic cell lines with a finite life. The angiogeneic reaction evoked by human bladder epithelium cell lines showed cell dose- and time-dependence; but it was unrelated to the growth potential of the cultured cells. Two "spontaneously" altered sarcoma-producing murine cell lines showed a higher angiogeneic activity than tumorigenic human bladder epithelial cells. The angiogeneic response to these two murine cell lines was unrelated to morphological signs of transformation and to differences in growth rate, serum requirement, saturation density, anchorage dependence, and isoimmunizing properties.

Tumor cells display the ability to produce the tumor angiogenesis factor (1) which induces formation of new blood vessels around the tumor. The increase in vascularization promotes the growth of a tumor (see Ref. 2), and neoplastic progression is accompanied by a rise in angiogeneic activity (3, 4). The intensity of angiogenesis induced by tumors varies with the histological type of the tumor (5).

Tumor angiogenesis factor obtained from various sources has been tested in many *in vivo* and *in vitro* assays (2). Angiogenesis evoked *in vivo* by live cells is a more complex process which, however, in spite of its complexity may be used as a rather simple *in vivo* assay for the quick estimation of the angiogeneic properties of injected cells.

Observations by Chodak *et al.* (6–8) and by Tatematsu *et al.* (9) indicate that angiogeneic activity may serve as a marker of neoplastic and preneoplastic lesions of the urothelium. These results may be useful in studies of the influence of chemical carcinogens on human bladder cells propagated *in vitro*. Such studies have been conducted for some time in our laboratory (10, 11). In order to establish reliable criteria of malignant transformation comparative studies were made of several nonmalignant and malignant cell lines. Using tumorigenicity in nude mice (12) as the criterion of malignancy, these cell lines have been allocated to one of four groups: Nonmalignant cells of normal origin, nonmalignant cells of tumor origin, malignant cells of normal origin, and malignant cells of tumor origin.

The comparison includes studies of the ability of the cells to invade cocultured fragments of embryonic chick heart (13–15) and other more traditional signs of transformation. The results will be described in detail elsewhere. The most striking finding was the complete correlation between tumorigenicity in nude mice and invasiveness *in vitro* irrespective of the origin of the cells. All other parameters studied have so far not yielded more than suggestive evidence of malignant transformation.

The aim of the present investigation was to test whether the angiogeneic activity of *in vitro* propagated human bladder cells injected into

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irradiated ST/a mice might serve as a marker of tumorigenic properties. For this purpose we used a modification of Sidky and Auerbach's *in vivo* assay for lymphocyte induced angiogenesis (16).

Materials and Methods. *Cell lines.* Ten established cell lines (see Table I), originating from human uroepithelium, were grown *in vitro* under the standard conditions as described previously (12–14). The medium consisted of Fib 41 (17) supplemented with seven nonessential aminoacids, 10% fetal calf serum, and antibiotics.

Six of these cell lines were derived from transitional cell carcinomas (TCC), while four came from histologically normal bladder mucosa. Eight cell lines showed "infinite growth transformation," and six were tumorigenic in nude mice, and invasive *in vitro*. Two of the six TCC explants produced a tumorigenic (Hu 961a, Hu 1734a) as well as a nontumorigenic (Hu 961b, Hu 1734b) cell line. Subcultures (HCV29T, Hu 609T) of two cell lines derived from histologically normal bladder mucosa showed apparently spontaneous malignant transformation during propagation *in vitro* (12). One of them (HCV29T) might have been initiated *in vivo*. This cell line was established by J. Fogh at the Sloan-Kettering Institute (18). It originated from the explanted biopsy of histologically normal bladder mucosa from a patient who had previously been irradiated because of TCC. The possibility that the apparently spontaneous transformation of the Hu 609 cell line might be due to cross-contamination with other cells has previously been discussed (12). Although it seems unlikely, the possibility cannot entirely be excluded.

Two murine cells, ST-L1 and ST-L22, were also studied. Both lines were originally fibroblastic cell lines established in the Fibiger Laboratory from explants derived from the lungs of adult ST/a mice. After 1–6 months of cultivation *in vitro* these cell lines underwent apparently spontaneous malignant conversion. Both became tumorigenic in newborn ST/a mice and both were able to invade and destroy fragments of embryonic chick hearts *in vitro*. However, the ST-L1 cells differed from the ST-L22 cells by changes in morphology (round cell transformation), growth pattern and by the development of strong isoimmunizing properties (15, 19). In immunoincompetent recipients they produced tumors which grew more rapidly than tumors produced by ST-L22 cells (19).

All cultures to be used in the angiogenesis assay were trypsinized in 0.25% trypsin + EDTA, rinsed three times with culture medium and finally twice with Fib 41.

Mice. For the *in vivo* assay 6-months-old female mice of the inbred ST/a strain were used. In order to prevent rejection of the human cells and the isoimmunizing ST-L1 cells the recipients were pretreated with whole-body irradiation, 7 Gy (700 rad) given by a Siemens Stabiliplan apparatus (0.95 Gy/min in air; 0.5 mm Cu) 2 hr before cell injection. Irradiation of the recipient has also been shown to enhance the angiogeneic reaction (16, 20).

Angiogenesis assay. The mice were anaesthetized with ip injection of Epontol (0.4 ml per mouse), and both flanks were shaved with an electric clipper. They were then injected intradermally with test cells suspended in 0.1 ml of Fib 41. To all cell suspensions 4–5 droplets per milliliter of trypan blue (1:1000) were added to facilitate the subsequent finding of the injection site. Each recipient mouse was injected with up to six different cell lines. Each kind of inoculum was injected at different flank locations on different mice to avoid the possible effects of the background vascularization of different skin areas on the intensity of angiogeneic reaction (21). After 3 days the recipient mice were killed by cervical dislocation, their skin was dissected from the underlying muscles and its inner surface was inspected under the dissecting microscope (Wild-Herrebrug, magnif. ×30). New blood vessels connected with the injection site were counted according to the morphologic criteria proposed by Sidky and Auerbach (16). All countings were blind and performed by the same person (MK).

Angiogeneic potency of human bladder epithelial cell line cells. The ability to induce angiogenesis was tested by the injection of 0.5 $\times 10^6$ viable cells of each human bladder epithelium cell line. To control the specificity of the angiogeneic reaction, lymph node cells of ST/a mice were used as negative controls as described previously (22). These cells are a potent source of angiogeneic factors when tested in an incompatible system (16), but

when injected into syngeneic hosts they do not evoke angiogenesis.

Cell dose- and time-dependence of human bladder cell line-induced angiogenesis. The cells of two highly angiogeneic cell lines, Hu 1703He and Hu 609 T, were injected in doses of 0.125, 0.25, and 0.5 × 10^6 cells per single injection and angiogenesis was evaluated on the second and third day after injection. In additional experiments, the cell dose- and time-dependence of angiogenesis was also established for murine ST-L1 and ST-L22 cells.

Angiogeneic potency of cells from confluent and log phase cultures. Both human and murine cell lines were grown *in vitro* under standard conditions to reach confluence. The cells were trypsinized within 24 hr after reaching confluence and used in the angiogenesis assay. In parallel cultures the same cells were grown at lower concentrations, providing cultures in the experimental growth phase at the termination of the experiment. These cells were used in the angiogenesis assay simultaneously with the cells from confluent cultures.

Angiogeneic potency of human bladder cell line cells irradiated in vitro. To test whether the ability to proliferate might influence the angiogenesis-inducing capability of human bladder cell lines, two cell lines were irradiated *in vitro* with a high dose of X-rays. The cells were trypsinized and rinsed as described above and placed in duplicate in plastic 5-cm petri dishes, each dish receiving 6 × 10^6 cells in 4 ml of Fib 41. The cells were then irradiated with a dose of 20 Gy (2000 rad) and subsequently injected into X-ray-immunosuppressed mice at a dose of 0.5 × 10^6 per injection. Cells from control groups were processed in the same way except irradiation. After 2–3 days the angiogenesis induced by the irradiated and nonirradiated cell lines was estimated.

Results. *Angiogeneic potency of human bladder epithelial cell lines.* The majority of human bladder epithelial cell lines tested showed angiogeneic activity when injected into mice pretreated with whole-body irradiation. The cell lines differed in their ability to evoke angiogenesis, but only two of them (Hu 961b and Hu 1767) displayed angiogeneic potencies which did not differ significantly from that expressed by control syngeneic lymphoid cells (Table I). These two cell lines, one derived from TCC and the other one from normal bladder mucosa, were both nontumorigenic, noninvasive *in vitro,* and did not show signs of infinite growth transformation.

The strongest reaction was seen after the

TABLE I. ANGIOGENEIC POTENCY OF HUMAN BLADDER CELL LINES ORIGINATING FROM MALIGNANT AND NORMAL UROTHELIUM[a]

Cell line[b]	Origin	Infinite growth	Tumorigenic[c]	Angiogenesis	Number of injections
Hu 549	Tumor	Yes[d]	Yes	13.67 ± 0.98	40
Hu 1703 He	Tumor	Yes	Yes	13.49 ± 0.83	43
Hu 609 T	Normal	Yes	Yes	13.04 ± 0.83	49
HCV 29 T	Normal	Yes	Yes	12.28 ± 1.27	25
Hu 961 a	Tumor	Yes	Yes	12.02 ± 0.92	30
Hu 1734 a	Tumor	Yes	Yes	11.33 ± 0.96	21
Hu 1734 b	Tumor	Yes	No	11.00 ± 1.00	23
HCV 29	Normal	Yes	No	9.73 ± 1.09	26
Hu 961 b	Tumor	No[e]	No	6.60 ± 0.73	10
Hu 1767	Normal	No	No	4.80 ± 0.73	5
ST/a lymph node cells (control)				5.40 ± 0.53	30

[a] Angiogenesis expressed as a mean ± SE of newly formed blood vessels per single injection site, estimated on the third day after the intradermal inoculation of 0.5 × 10^6 cells into ST/a female mice preirradiated with a single total body dose of 7 Gy.

[b] The HCV 29 cell line was established at the Sloan-Kettering Institute by J. Fogh (18). All other cell lines were established in our laboratory by M. Vilien (12) and B. Christensen.

[c] Tested by the sc injection of 10^7–10^8 cells into nude mice.

[d] Propagated for more than 75 passages in vitro. Doubling time ≤2 days.

[e] Tested in the 15th to 25th passage. Hu 1767 stopped growing in the 29th passage. Hu 961 b shows decreasing growth rate with a passage interval increasing from originally 1 month to 2 months in the 38th passage.

injection of tumorigenic cell lines. The differences in angiogeneic potency between tumorigenic and nontumorigenic cell lines were significant at $P < 0.05$ as estimated by nonparametric Kruskall–Wallis test (23).

The angiogeneic potency of human bladder epithelial cell lines grouped according to origin and tumorigenicity is shown in Table II. The average angiogeneic response to all four groups was significantly higher than the response to ST/a lymph node cells.

Tumorigenic cell lines derived from bladder neoplasms or from *in vitro* transformed urothelium of nontumor origin induced angiogeneic reactions of the same intensity, which was significantly higher than that evoked by nontumorigenic cell lines of tumor as well as of nontumor origin. The two groups of the latter cell lines did not differ significantly. All comparisons were done with one-way analysis of variance and further comparison involved the Duncan's new multiple-range test at $P < 0.05$ (24).

Cell dose- and time dependence of human bladder cell line-induced angiogenesis. The results are illustrated in Fig. 1. The cell-dose dependence of angiogenesis was estimated within the range of $0.125–0.5 \times 10^6$ cells per single injection, and the regression lines were fitted by the least-squares method at the 0.95 level. The cell-dose dependence was more marked when tested on the third day after cell injection. Similar results were obtained with Hu 609T, ST-L1, and ST-L22 cells.

Angiogeneic potency of cells from confluent and log phase cultures. Neither human nor murine cells from cultures in the logarithmic growth phase or from confluent cultures showed any significant ($P > 0.05$ by Student's

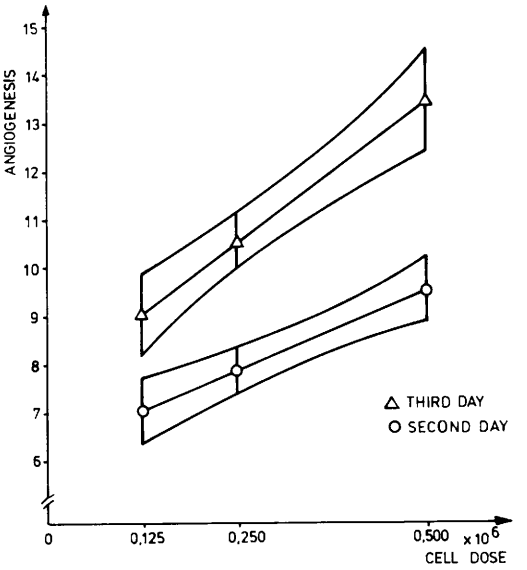

FIG. 1. Cell dose and time dependence of the angiogeneic response to human bladder carcinoma cell line cells (Hu 1703He) injected intradermally into irradiated (7Gy) ST/a female mice. Angiogenesis expressed as the mean number ±SE of newly formed blood vessels counted 2–3 days after injection.

t test) differences in their angiogeneic potency when injected into irradiated ST/a mice (Table III). In the same experiment it was found that in spite of the previously demonstrated significant difference between the growth rate of ST-L1 and ST-L22 cells in immunoincompetent recipients (19), no significant difference could be demonstrated between the angiogeneic potency of these two cell lines.

From Fig. 2 it is seen that both HU 1703 He and Hu 609 T exhibit density inhibition at the stage of confluence. Similar curves have been demonstrated for ST-L1 and ST-L22 (19).

TABLE II. ANGIOGENESIS EVOKED BY HUMAN BLADDER CELLS OF TUMOR OR NORMAL ORIGIN THAT WERE SHOWN TO BE TUMORIGENIC OR NONTUMORIGENIC IN NUDE MICE[a]

Origin of cell line	Tumorigenicity	Angiogenesis mean ± SE	Number of injections
Tumor	Tumorigenic	12.87 ± 0.475	134
Tumor	Nontumorigenic	9.66 ± 0.87	33
Normal	Tumorigenic	12.77 ± 0.70	74
Normal	Nontumorigenic	8.93 ± 0.98	31
ST/a lymph node cells		5.40 ± 0.53	30

[a] Angiogenesis expressed as a mean number ± SE of newly formed blood vessels per single injection site, evaluated on the third day after the intradermal injection of 0.5×10^6 cells into ST/a female mice preirradiated with a single body dose of 7 Gy.

TABLE III. THE ANGIOGENEIC POTENCY OF CELLS FROM CONFLUENT AND LOG PHASE CULTURES[a]

Cell line	Angiogenesis	
	Confluent cultures	Log phase cultures
Hu 1703 He	12.93 ± 0.82 (15)[b]	13.13 ± 0.96 (15)
	9.50 ± 0.67 (12)	9.50 ± 0.79 (12)[c]
Hu 609 T	13.62 ± 1.59 (8)	12.12 ± 1.06 (8)
ST-L1 (murine)	19.10 ± 1.54 (10)	18.80 ± 2.48 (10)
ST-L22 (murine)	17.00 ± 1.27 (9)	17.80 ± 2.15 (10)

[a] Angiogenesis expressed as a mean number ± SE of newly formed blood vessels per single injection site on the third day post-intradermal injection of 0.5×10^6 cells into ST/a female mice preirradiated with a total body dose of 7 Gy.

[b] Number of injections in parentheses.

[c] Estimated on the second day after cell injection.

TABLE IV. ANGIOGENESIS EVOKED BY 0.5×10^6 IRRADIATED OR NONIRRADIATED CELLS[a]

Cell line	Angiogenesis	
	Irradiated cells	Control cells
Hu 1703 He	14.50 ± 1.35*	13.17 ± 1.41
Hu 609 T	12.58 ± 0.77	13.17 ± 1.35

Note. The differences between irradiated and nonirradiated cells are not different at $P < 0.05$.

[a] The cells were irradiated *in vitro* with a dose of 20 Gy given 2 hr before injection into whole-body irradiated (7 Gy) female ST/a mice. Angiogenesis expressed as the mean number ± SE of newly formed blood vessels per single injection site evaluated on the third day after intradermal injection.

[b] Means of twelve injections.

Angiogeneic potency of human bladder cell lines irradiated in vitro. In order to test whether cell division after injection into ST/a mice might have any influence on the angiogenesis assay, two bladder cell lines (Hu 1703 He, and Hu 609 T) were irradiated with 20 Gy 2 hr before injection into whole-body irradiated recipients. As shown in Table IV and Fig. 3 irradiation of these cells did not influence the angiogeneic response measured 3 days after the injection.

However, after 2 days (Fig. 3) the angiogeneic response to 0.25–0.50 × 10^6 irradiated Hu 1703 He cells was higher than to nonirradiated controls.

Discussion. The results show that epithelial cells derived from human bladder cell lines propagated *in vitro* are able to evoke angiogenesis when injected into X-ray-immunosuppressed mice. The reaction shows cell-dose dependence, and it is higher on the third than on the second day after cell injection. The dose–response relationship is similar to that observed in lymphocyte-induced angiogenesis assays when human or murine lymphocytes are used (16, 22, 25).

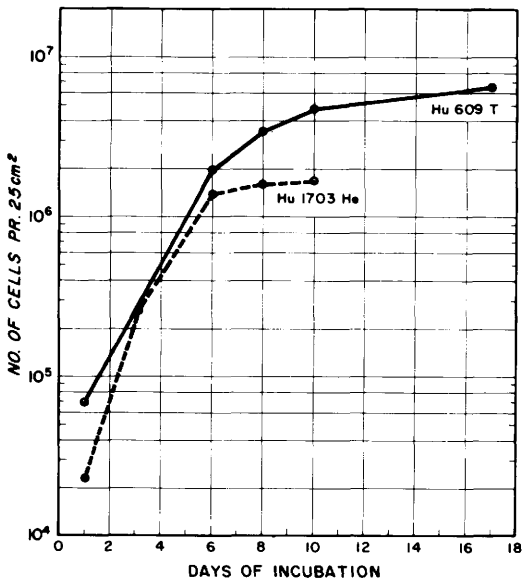

FIG. 2. Growth of tumorigenic human urothelial cells counted in Bürker–Türk's hemocytometer.

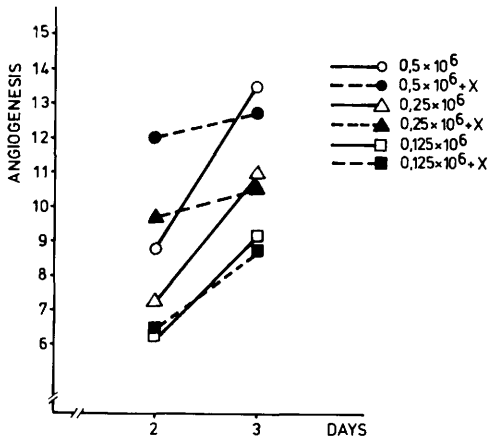

FIG. 3. Influence of *in vitro* irradiation (20 Gy) on angiogeneic activity of human bladder carcinoma cell line (Hu 1703He) injected intradermally into irradiated (7 Gy) ST/a female mice. Angiogenesis expressed as the mean number of newly formed blood vessels scored on the second and the third day after the injection.

Eight out of a total of 10 human bladder epithelium cell lines tested in the present study evoked an angiogeneic response which was significantly higher than the response to ST/a syngeneic lymph node cells. However, the intensity of angiogenesis evoked by the same number of cells from different human bladder epithelium cell lines differed.

The highest angiogenesis-inducing ability was expressed by those cell lines that were shown to be tumorigenic in nude mice. The tumorigenic lines did not differ significantly in their angiogeneic potency, irrespective of their origin. Nontumorigenic lines of normal as well as of tumor origin were less angiogeneic than the tumorigenic lines. Two of them—one of tumor origin and one of normal origin—evoked an angiogeneic response which did not differ significantly from that obtained by the injection of control lymph node cells. Thus, angiogenesis was found to correlate with the tumorigenicity of the cells but not with their origin.

As already mentioned a similar correlation, irrespective of the origin of the cells, has been observed between tumorigenicity and invasiveness *in vitro.*

The finding that three out of five nontumorigenic cell lines—two of normal and one of tumor origin—showed moderate angiogeneic activity, raises the question whether these lines might have undergone partial transformation *in vitro.* The hypothesis is supported by the fact that all three cell lines showed infinite growth transformation in contrast to the two nontumorigenic cell lines showing an angiogeneic activity which did not differ significantly from that of the control lymphocytes.

Transformed cells which are nontumorigenic by standard tests, may in special model situations produce tumors, and such cells are angiogeneic (26). The fact that the production of tumors in nude mice by human TCC cells requires a cell dose as high as 10^7–10^8 cells shows that the tumorigenicity test is not a very sensitive one.

The nontumorigenic HCV29 cell line which was derived from previously irradiated, but morphologically normal human bladder epithelium, showed a moderate, but statistically significant increase in angiogeneic activity as compared to ST/a lymph node controls. This cell line underwent apparently spontaneous neoplastic alteration during propagation *in vitro* (12) and developed at the same time invasive properties and a further increase in angiogeneic potency. As already stated it cannot be excluded that these cells were initiated during X-ray treatment *in vivo.* Thus, at the time of explantation they might have been in a preneoplastic state which may account for their angiogeneic activity.

During the malignant progression of mammary tissue, there is a concomittant increase in angiogeneic activity of the tumor tissue (3, 4). The question arises whether such increase of angiogeneic activity occurs in the course of malignant conversion *in vitro.* Ziche and Gullino (27) have observed that angiogeneic capacity appeared in cultures of human, murine, and hamster cells long before a neoplastic transformation could be demonstrated by transplantation. It is conceivable that angiogenesis is an early step toward malignant transformation. Our observations could possibly be interpreted as a sequence of reactions in which infinite growth transformation and angiogenesis represent an initial event which is later followed by the development of invasiveness (15, 19) and finally of tumorigenicity. However, the interdependence between angiogenesis and oncogenesis remains to be proven.

The two murine cell lines, ST-L1 and ST-22, which were included in the present investigation, represent two types of apparently spontaneous malignant alteration *in vitro,* one which is accompanied by morphological and immunological changes as well as changes in growth potential and growth pattern (ST-L1), and one which does not show these signs of transformation (ST-L22). However, both of them were tumorigenic in immunoincompetent recipients (ALS treated newborn ST/a mice and adult nude mice) at a TD_{50} dose of 4.4×10^2–2.3×10^3 cells (19), invasive in cocultures with fragments of embryonic chick hearts (15, 19, 28), and highly angiogeneic. Thus, like tumorigenicity and invasiveness, angiogenesis seems to be a more general sign of neoplastic alteration than the traditional *in vitro* criteria of transformation.

The association of a high angiogeneic potency of a given cell line with a high tumorigenic potential in nude mice is to be expected since cells that efficiently induce new blood

vessels in their vicinity should have a much better chance to survive and to grow than cells with only a weak angiogeneic effect. It has been shown that tumor cells and the endothelial cells form a highly related ecosystem in neoplasms and the growth of endothelium determines the growth of tumor cells (29).

After sc inoculation into immunoincompetent recipients the ST-L1 cells produced sarcomas which grew more rapidly than sarcomas derived from ST-L22 cells (19). Thus, the ST-L1 cells might have been expected to possess a higher angiogeneic potential. However, this was not the case. The difference between the growth rate of ST-L1 and ST-L22 tumors *in vivo* probably reflects a difference between the growth capacity of these two cell lines which was also demonstrable under *in vitro* conditions (19).

The absence of any significant difference between the angiogeneic potency of the rapidly growing ST-L1 cells and the more slowly growing ST-L22 cells shows that the angiogeneic capacity and the rate of cellular proliferation are not necessarily related to one another. This is supported by the finding that log phase cultures and confluent cultures of the same cell line show identical angiogeneic activities. Furthermore, preirradiation of the injected cells did not reduce their angiogeneic effect.

Loss of density inhibition is generally considered characteristic of malignant cells. However, it has previously been demonstrated (26) that density inhibited cell lines may produce angiogenesis factor (TAF), and it was suggested that the production of TAF is an early event in the cell transformation process that precedes the loss of density inhibition of growth *in vitro.*

Also the present cell lines showed density inhibition when they reached the stage of confluence. Round cell transformation was accompanied by an increase in saturation density, but even the ST-L1 cells showed density inhibition (19). Thus, unrestrained cell division does not seem to be an obligatory step in the process of neoplastic transformation, but it may possibly be a later manifestation of progression.

The experiments with irradiated tumor cells are in agreement with previous reports according to which irradiation does not destroy the angiogeneic potency of tumor tissue (30) or lymphocyte suspensions (20). The reason for the higher initial response to irradiated cells as compared to nonirradiated remains to be elucidated. Maybe the tumor angiogenesis factor is more readily released from the irradiated cells. The fact that irradiated cells are still capable of producing angiogeneic factors—at least a short time after irradiation—suggests that mixtures of weakly tumorigenic cells and irradiated cells of high angiogeneic potency may facilitate the growth of the former in a suitable host and thus increase the sensitivity of tumorigenicity tests.

The specificity of the angiogeneic reaction deserves some comment. In an allogeneic system murine lymph node cells evoke an angiogeneic reaction which is not seen when the cells are injected into syngeneic recipients (16). In the present study immunological factors do not seem to have played a role. The ST/a recipients were whole-body irradiated, and the angiogeneic response to isoimmunizing ST-L1 cells of ST/a origin was the same as to syngeneic, nonisoimmunizing ST-L22 cells. Furthermore, the response to xenogeneic human bladder cells was significantly lower than the response to the two ST/a-derived murine cell lines.

The difference between the antigenic properties of ST-L1 and ST-L22 cells are mainly due to the three times higher concentration of MuLV structural protein gp 70 in the morphologically transformed ST-L1 cells (31), but according to the present results this did not cause a significant increase in the angiogeneic activity of these cells as compared to the morphologically untransformed ST-L22 cells.

The influence of virus on angiogenesis has been discussed by previous authors. Thus, it has been found (26) that SV40 transformed BALB/c 3T3 are angiogeneic, but the activity was 10–20 times less than that of human meningioma cells. Ziche and Gullino (27) have reported that MuMTV infection of C57BL/6 mammary epithelial cells failed to enhance the angiogeneic response to a sufficiently high frequency to convincingly indicate that MuMTV played a decisive role in inducing angiogeneic capacity of infected cells.

It is conceivable that the difference between the response to murine and human cells is related to more specific interactions of en-

dothelial receptors with angiogeneic factors produced by the syngeneic cells than those produced by xenogeneic cells, but one may also speculate whether sarcoma cells in general have a higher angiogeneic potential than carcinoma cells.

Apart from quantitative differences between the angiogeneic reactions evoked by murine and human cells in irradiated ST/a mice, the reactions were very similar with regard to dose–response relationship, time dependence, correlation with tumorigenicity and invasiveness, and independence of the rate of cellular proliferation. Thus, the tentative conclusion to be drawn from the present investigation seems to be that the angiogeneic response of irradiated mice to human and murine cell lines propagated *in vitro* may possibly predict the development of neoplastic transformation or serve as a tumor marker even in slowly growing cultures which do not show morphological signs of transformation, immunological changes, increased saturation density, ability to grow in semisoft agar, and other changes suggestive of malignant alteration. If this conclusion is substantiated investigations of the production of tumor angiogenesis factor may be useful in studies of carcinogenesis in cultures of human and other cells. Such studies might help to clarify the interdependence, if any, between angiogenesis and oncogenesis.

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