Difference in the Growth-Regulating Effect of Syrian Hamster Fibroblasts in Early and Late Periods of Embryogenesis on Tumor Cells of Various Degree of Malignancy

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Embryonal fibroblast cultures are as a rule used as normal cells in studies of normal cell growth-regulating activity towards tumor cells [16,17,19]. This gives rise to a number of problems, one of which concerns the degree to which embryonal cells can regulate proliferation, sinc4e the level of their own proliferative activity is very high in comparison with that of the majority of adult organism cells. Another question is whether the pattern of tumor cell interaction with embryonal fibroblasts depends on embryonal age; in other words, does the system of regulation of proliferative processes, specifically, the system of tumor cell proliferation, change in the course of embryonal development? To solve these problems, we used Syrian hamster embryomal fibroblast cultures in the early and late periods of embryonal development. The objects of growth-regulating activity of these fibroblasts in mixed cultures were *in vitro* transformed hamster embryonal fibroblasts and a number of their *in vivo* selected variants differing in tumorigenic and metastatic activities.

MATERIALS AND METHODS

The embryonal fibroblast culture was prepared by trypsin treatment of hamster embryos during various periods of embryonal development. Early hamster embryo cultures were prepared from 7-9-day-old embryos, while late ones were prepared from fetuses directly before birth (at 17-18 days). The cells were used in experiments during the second-fifth *in vitro* passages when the fibroblasts were the predominant type of cells in the cultures.

The following tumor cell strains were used in the experiments: *in vitro* spontaneously transformed Syrian hamster embryonal fibroblasts HETR; HETR cell variants of different degrees of malignancy selected *in vivo* - HETR-128, HETR-MLN-6, HETR - MLN-8, HETR - 75/18, and HETR - 83/20; cell strains obtained by Syrian hamster embryonal fibroblast transformation by the Rous sarcoma virus (RSV), Schmidt-Ruppin strain: HET-SR, HET-SR-1, and HET-SR-10. The characteristics of the strains are presented in Table 1.

Eagle's medium with lactalbumin hydrolysate in a 1:1 ratio, 10% bovine serum, and 0.03 mg/ml glutamine were used for culturing the embryonal cells, the HETR strain, and its variants. For the RSV-transformed cells F-12 medium with 10% bovine serum was used. Eagle's medium with the additions described above and with 5 mM HEPES buffer and 0.08 mg/ml gentamicin was used in the experiments.

Linbro (Flow) 96-well round-bottom plates were used. Early and late embryonal cultures were removed from flasks with versene solution, diluted

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with experimental medium to attain the required concentration, and poured into the wells, 5×10^4 cells per well. Approximately 20 hours later, when a monolayer had formed, the plates with cells were irradiated with a Stebel instrument for 8 min in a dose of 10,000 rad. Tumor cell suspension prepared beforehand were then added to the wells, 1×10^4 cells per well. The cells were cocultivated for 4 days with daily visual monitoring and daily replacement of half the well volume of medium under standard conditions (37°C and 5% CO_2).

Tumor cell proliferative activity was assessed by the incorporation into the cell nuclei of tritium-labaled thymidine (3-HTdR, 0.25 μCi/well, 5Ci/mM diluted with cold thymidine, 10 μl/ml), which was added to the medium for 20 h. The cells were then washed, removed from the plates with versene solution, and placed on fiberglass filters with a cell harvester (LKB, Sweden); the amount of incor-porated label was scintillated by a β-counter (cpm).

The stimulating or inhibiting effect of the embryonal cells on tumor cells was assessed by the formula:

$$\%=\frac{\text{cpm TC-(cpm TC/HE - cpm HE)}}{\text{cpm TC}}$$

where TC - tumor cells, HE - hamster embryo cultures, cpm TC - label incorporation in control tumor cells in the absence of contact with hamster embryo cultures, cpm TC/HE - label incorporation in tumor cells in contact with irradiated hamster embryo cultures, and cpm HE - label incorporation in irradiated hamster embryo cells.

RESULTS

The time course of proliferation of tumor cells of various degrees of malignancy and of cells transformed by various agents during contact of these cells with a solid monolayer of early or late embryonal fibroblasts was followed up for 4 days of cocultivation and compared to intact tumor cell proliferation.

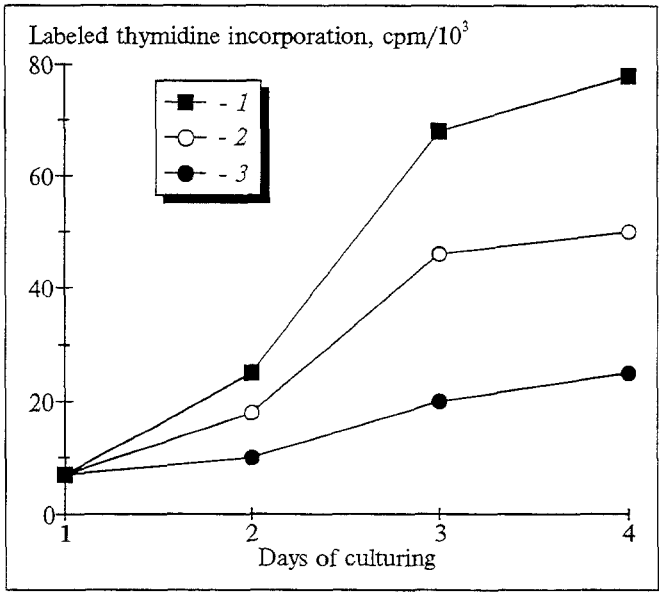

Fig. 1. Proliferative activity of HETR cells during contact with early and late hamster embryo cell cultures. *1*) HETR; *2*) HETR in contact with late hamster embryo cultures; *3*) HETR in contact with early hamster embryo cultures. Each point represents the mean value of results in 4 similar wells.

Tumor cells in a dose of 1×10^4 cells per well were embedded in a monolayer of irradiated early and late hamster embryo fibroblasts seeded in a dose of 5×10^4 cells per well. Tumor cell proliferative activity was assessed by labeled thymidine incorporation. Early and late hamster embryo fibroblasts seeded in such a dose virtually did not proliferate [1]. Irradiation of normal cells before the experiment helped minimize 3-HTdR incorporation and reduce it to the background level and also ruled out possible changes of both the early and late cultures' proliferative activities resulting from contact with tumor cells. From the data of label incorporation in tumor cells we plotted tumor cell growth curves (Figs. 1-3). The differences in thy-

TABLE 1. Characteristics of Tumor Cell Strains

Strain	TGA, log TrD_{50}	Experimental metastatic activity, min.dose	Spontaneous metastatic activity, + or -
HETR	1.7–2.4	$\gg2.0\times10^6$	–
HETR–128	–"–	$>3.0\times10^6$	–
HETR–162	–"–	1.0×10^6	–
HETR–75/18	0.7–1.2	10^4-10^5	+ +
HETR–MLN–6	1.5–1.7	–"–	+
HETR–MLN–8	1.2–1.4	–"–	+ + +
HETR–83/20			+ + +
HET–SR	0.5–1.8	–"–	–
HET–SR–1	–"–	–"–	+ + +
HET–SR–10	–"–	–"–	+ +

Note. TGA was assessed in the quantitative transplantation test as the log of 50% of the transplantation dose.

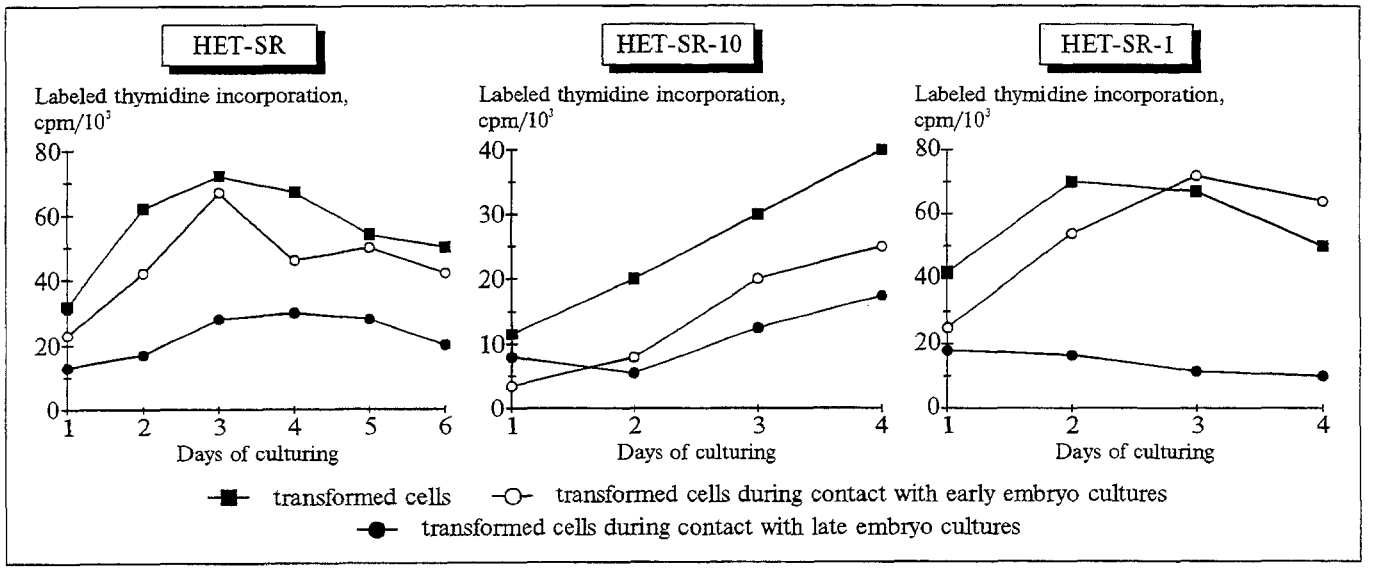

Fig. 2. Proliferative activity of Rous sarcoma virus – transformed cells during contact with early and late hamster embryo cell cultures.

midine incorporation levels by various strains are most likely due not to actual differences in the proliferative activities of cells of different strains but to specificities of labeled thymidine processing.

The effect of early and late hamster embryo fibroblasts on tumor cell proliferative activity was calculated by the above formula. Inhibiting (-) or stimulating (+) effects of these cultures are shown in Table 2. The data indicate that the effects of early and late cultures on the proliferative activities of various tumor cell strains were different. The inhibitory effect of early cultures was stronger than that of late cultures in all the tested tumor cell strains. In addition, with highly malignant tumor cell strains early and late hamster embryo cells differed in their capacity for growth-stimulating and/or growth-inhibiting action. The proliferative activity of HETR spontaneously transformed strains was noticeably inhibited over the whole follow-up period during their contact with both early and late embryo cells, the inhibitory effects of both increasing from 5-20% on days 1-2 to 60% on day 4 of the experiment. Moreover, the data of this study as well as our previous findings (when we used only early hamster embryo fibroblasts) [1] demonstrated that the inhibitory effect of early embryonal cells had a tendency to increase with time to the point of complete arrest of HETR cell proliferation, whereas the similar effect of late embryonal cultures was somewhat attenuated by day 4 in comparison with days 2-3 of the experiment (Fig. 1).

The effects of early and late embryonal cultures on the proliferative activity of the HETR

TABLE 2. Inhibiting (−) or Stimulating (+) Effect (in %) of Syrian Hamster Early and Late Embryonal Fibroblasts on Proliferative Activity of Tumor Cells of Various Degrees of Malignancy as Shown by Results of Individual Experiments

Cocultivation time, days	Tumor cell strain																	
	HETR		HETR-128		HETR-MLN-6		HETR-MLN-8		HETR 75/18		HETR 83/20		HET-SR		HET-SR-1		HET-SR-10	
	EEF	LEF	EEF	LEF	EEF	LEF	EEF	LEF	EEF	LEF	EEF	LEF	EEF	LEF	EEF	LEF	EEF	LEF
1	+12	−2	−58	+25	−18	−10	−7	+40	−26	+20	+15	+52	−60	−28	−70	−57	+30	+32
	−64	−32	−86	−48	−54	−11	−27	−5	−33	−64			−53	−36	−30	−81	−57	−43
	−44	−17																
2	−52	−32	−65	−42	−56	−41	−19	+34	−56	+20	−17	−12	−75	−55	−67	−60	21	−28
	−95	−54	−77	−56	−54	−38	−35	+35	−56	+2			−73	−39	−69	−61	−77	−24
	−60	−43																
3	−69	−32	−85	−52	−77	−24	−60	−13	−43	−31	−22	+30	−70	−56	−31	−24	−31	−24
	−79	−45	−75	−50	−71	−11	−40	+20	−65	−33			−65	−10	−60	−1	−83	−1
	−76	−38																
4	−72	−12	−77	−61	−72	−2	−41	+20	−51	+12	−30	−4	−29	−30	+15	+23	−27	−17
	−90	−55	−79	−72	−52	−21	+7	+35	−69	−34			−29	−55	−57	−40	−77	+20
	−82	−20					−16	−8	−40	+131								

Note. EEF: early embryonal fibroblasts; LEF: late embryonal fibroblasts.

malignant variant, HETR-128, did not differ in principle from their effects on HETR. The difference in the effects of the early and late embryonal cultures was somewhat less pronounced over the course of the experiment: 20-40% of the proliferative activity of HETR-MLN-6, a moderately malignant HETR variant, was inhibited over the course of the experiment (Fig. 3), particularly so in contact with the early cultures, whereas late cultures exerted a weak inhibitory effect. A different picture was seen with the proliferative activities of *in vivo* selected highly malignant tumor cells during their contact with early and late hamster embryonal fibroblasts. Exposure to early embryonal cultures markedly reduced the proliferative activity of these strains during four days of the follow-up; in some cases (HETR-MLN-8) this effect weakened in days and virtually disappeared by the end of the experiment. Late embryonal cultures had no noticeable growth-inhibitory effect on the proliferative activities of these tumor cell strains; moreover, in the majority of cases a stimulating effect of these cultures on cells of such strains was observed.

Examination of the proliferative activty of *in vitro* RSV-transformed cells showed that their behavior was in many respects similar to that of HETR cells. The proliferative activity of these cells was inhibited by both early and late fibroblasts cultures, but in both cases there was a pronounced trend toward attenuation of the inhibitory effect by the end of the experiment. Moreover, in some experiments with HET-SR-1 and HET-SR-10 cells late cultures had a growth-stimulating effect and HET-SR-1 proliferative activity was increased when the cells were exposed to early embryonal cultures (Table 2, Fig. 2)

These data indicate that normal cells in various periods of embryogenesis differ in their ability to regulate proliferative processes in cell cultures. An embryo or fetus developing through various stages of growth, differentiation, and organo-

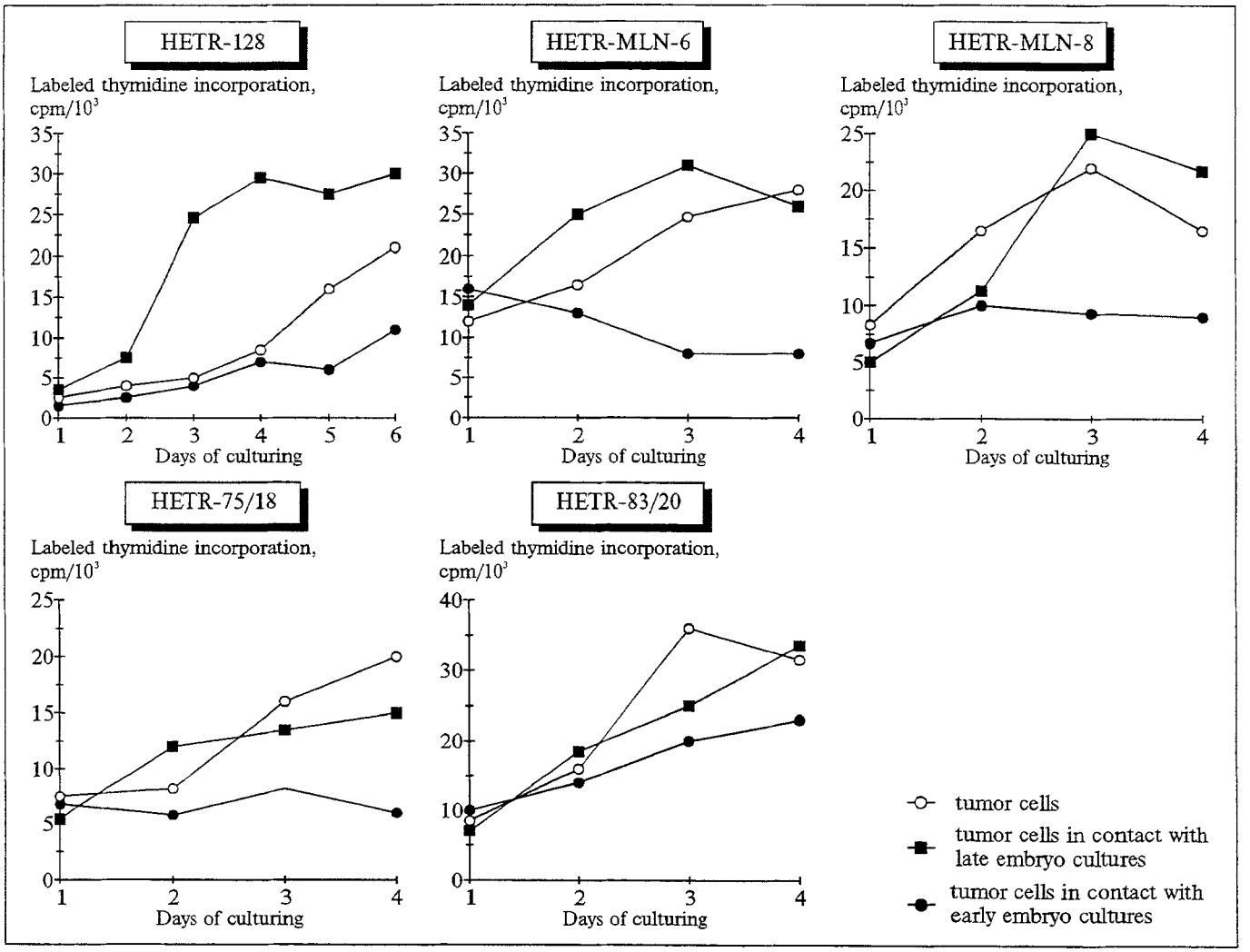

Fig. 3. Proliferative activity of various lines of *in vivo* selected tumor cells.

genesis behaves in some respects like a tumor. It invades maternal tissues [15] and trophoblast cells may migrate similarly to metastasizing tumor cells [2]. Moreover, protooncogene expression in developing embryo tissues is similar to the expression of a number of oncogenes in tumor tissue [7,10,18], but still, growth remains strictly regulated up to a certain level, resulting in the development of a normal organism. The conclusion that is drawn from all these observations is that during embryogenesis cell growth and invasiveness is stringently controlled. Some earlier findings confirm this viewpoint. For example, it has been shown that during the induction of differentiation processes a mechanism of cell death exists to eliminate cells not responding adequately to differentiation signals [12]. Moreover, there are descriptions of interesting phenomena pertaining to embryonal environmental control over malignant properties of tumor cells [6]. These results were obtained when tumor cells were injected into mouse blastocytoma. The eventual fate of the cells varied greatly - from complete elimination and normal fetal development to tumor phenotype expression during embryogenesis or after birth [9,11,13,14]. Nonetheless, the proportion of tumor cells in an embryo or a newborn mouse in chimera experiments was much lower in comparison with that of normal cells injected for control [8], this confirming the presence of a possible embryonal regulation of growth and tumor cell differentiation. The results of the present study uphold this view and, in addition, they demonstrate different efficacies of these processes during embryogenesis. This may be explained by different production of growth-regulating factors, of TGF_{β} for example [3], at the early and late stages of embryogenesis [5].

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