Brief Definitive Report

ACQUISITION OF HIGH METASTATIC CAPACITY AFTER IN VITRO FUSION OF A NONMETASTATIC TUMOR LINE WITH A BONE MARROW-DERIVED MACROPHAGE

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Although tumor heterogeneity (1) and progression (2) have important biological implications and therapeutic consequences, little is yet known about basic mechanisms of tumor diversification and variant generation. Somatic hybridization between tumor cells and normal host cells has been reported (3) to occur in vivo and could potentially contribute to the diversification and generation of highly malignant variants. To test this possibility we attempted the in vitro fusion of tumor cells with defined normal host cells such as macrophages, which are often present within growing tumors. We herein report on two highly metastatic hybrid lines independently obtained after in vitro hybridization of a nonmetastatic murine lymphoma line (Eb) (4, 5) with syngeneic bone marrow–derived macrophages. Interestingly, such high metastatic hybridomas were found to express a tumor antigen similar to that expressed by spontaneous in vivo derived high metastatic variants (ESb) (6–8) of the same tumor.

Materials and Methods

The two cell types to be fused were carefully selected. The lymphoma line was made thioguanine resistant (Eb^{TGR}), and thus HAT (hypoxanthine, aminopterin, thymidine)-sensitive, without mutagenesis, by growing it in gradually increasing concentrations of thioguanine up to 4 μg/ml, followed by cloning. G-banding caryotype analysis revealed that the clones were unstable and heterogeneous while the noncloned Eb^{TGR} line was stable, homogeneous, and identical in all eight marker chromosomes with the parental line Eb (8). As fusion partner for the Eb^{TGR} line we used in vitro differentiated, bone marrow–derived macrophages precultured for 15 d in L cell–conditioned medium. As described elsewhere (9), these cultures consisted predominantly of macrophages that were highly spread out and had phagocytic activity. Tumor macrophage fusions with polyethylene glycol (PEG) (Merck AG, Darmstadt, FRG) (1,000 mol wt; 45% in RPMI, pH 7.5, or in 0.15 M Hepes) were performed with a modified procedure (10). Fusion cultures consisted of Eb^{TGR}, macrophages, and PEG, while control cultures had either Eb^{TGR} cells and PEG, macrophages and PEG, or Eb^{TGR} cells and macrophages without PEG.

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Results and Discussion

From two independent fusion cultures of Eb^{TGR} cells and macrophages, two hybridomas (Eb-F1 and Eb-F2) were successfully established as HAT-resistant lines in tissue culture 40 d postfusion, while nothing was obtained from control cultures. The G1 DNA content of these lines was analyzed by a cytofluorograph after staining with 4,6-diamidino-1-phenylindole (DAPI). It was found to decrease with time from a hypotetraploid level at 40 d postfusion, representative of a slightly reduced hybrid, to a diploid (like Eb and ESb), and then hypodiploid at 60 d postfusion (both by Eb-F1 and Eb-F2). At this stage, where both lines appeared to be fairly well stabilized, they were cloned. Both the original lines and subclones were further investigated.

Evidence for the hybrid nature of the two cell lines, obtained by cytogenetic analysis of G-banded metaphase chromosomes (Fig. 1), consists primarily of the presence of normal chromosomes No. 12, which were never found in the parental lymphoma line, and which coexisted in the hybrids with the two abnormal forms of chromosomes 12 (one translocated, 12^+, and one deleted, 12^- [8]) of the lymphoma line. Apart from the additional normal chromosome 12, the hybrid lines carried several new marker chromosomes not found in the parental Eb^{TGR} cells. The high metastatic in vivo variant ESb did not contain a normal chromosome 12 and carried a marker chromosome with a fragment from 12 that was not found in Eb-F1 or Eb-F2 cells.

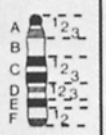
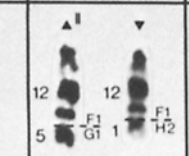
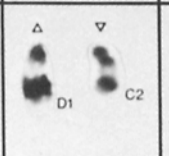
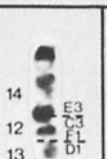
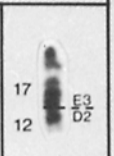
CHART OF NORMAL 12	NORMAL 12	12'+' FORMS t(12;5)	12'+' FORMS t(12;1)	12'−' FORMS 12d	12'−' FORMS 12dd	TRANSLOC. FRAGMENTS t(14;12;13)	TRANSLOC. FRAGMENTS t(17;12)
							
Eb	−	$1^{\blacktriangle}$		$1^{\triangledown}$		1	−
Eb^{TGR}	−	$1^{\blacktriangle}$		$1^{\triangledown}$		1	−
MACROPHAGE	2	−		−		−	−
Eb-F1	1	$1^{\blacktriangledown}$		$1^{\triangledown\triangle}$ ‡		−	−
Eb-F2	1	$1^{\blacktriangledown}$		$1\text{-}2^{\triangledown\triangle}$ §		−	−
ESb	−	$1^{\blacktriangledown}$		$2^{\triangle}$		−	1

FIGURE 1. Analysis performed on 25 G-banded metaphases per cell line, giving numbers of normal or abnormal No. 12 chromosomes found in 90–100% of the metaphases. No. 12^+ and 12^- forms appeared in two subtypes. Their presence in each cell line is indicated by the symbols ▲, ▼, and △, ▽, respectively. (‡) 70% of the metaphases with one and 30% with two chromosomes. (§) 50% of the metaphases with one and 50% with two chromosomes.

The new lines Eb-F1 and Eb-F2 were inoculated subcutaneously into syngeneic DBA/2 mice and compared with the parental Eb^{TGR} line for tumorigenicity and metastatic capacity (Fig. 2). Mice inoculated with the parental line developed large primary tumors and died after ~6 wk, without overt metastases. In contrast, mice inoculated with the tumor-macrophage hybridomas died after only ~10–14 d, with small primary tumors and metastases in liver, lung, and spleen. Similar results were obtained in three separate experiments and also with subclones Eb-F1F1 and Eb-F2C4 (Fig. 2), consistently demonstrating the acquisition of high metastatic capacity by the somatic cell hybrids. EB-F1 cells isolated from in vivo tumors were cytogenetically similar to those from tissue culture.

Next we investigated cell surface marker expression to compare the new in vitro generated variants with the original in vivo derived high metastatic variant ESb (Fig. 2). Tumor-associated transplantation antigens (TATA) were typed in vitro as described (6, 7, 11), using syngeneic, tumor-specific, cytotoxic T lymphocytes (CTL). This analysis clearly revealed that the in vitro generated high metastatic variants expressed a TATA different from that of the parental Eb^{TGR} cells and similar to that of ESb type cells (i.e., $TATA_2$). It was previously demonstrated (11) that the ESb TATA was a distinct marker of this cell line which was not expressed on any other syngeneic or allogeneic tumor line tested. Eb-F1 and Eb-F2 cells also showed, similar to ESb type cells, a high expression of Fc receptors (12), as detected in an EA rosette assay, while Eb^{TGR} cell expression was low. The pattern of expression of T lymphoid differentiation antigens (Thy-1, Lyt-1, Lyt-2) also changed on the hybrid lines, but not exactly the same way as on ESb type cells. While all three markers were expressed on Eb-F1 and Eb-F2 cells, Eb^{TGR} cells expressed predominantly Thy-1 and Lyt-2 while ESb cells expressed predominantly Lyt-1, as reported previously (13). Taken together, the profiles of marker expression (Fig. 3) show (*a*) the profound changes that took place after fusion of Eb^{TGR} cells with macrophages, and (*b*) the similarities to the changes seen in vivo during progression of Eb to ESb type cells.

Fig. 2 also shows the mortality curves from mice inoculated with Eb^{TGR} cells to which small numbers of ESb type cells had been added. A pronounced shift in the mortality curves was observed (Fig. 2) even when as few as 10 ESb cells were added to 10^6 Eb^{TGR} cells. This shows that the metastatic tumor cells could not have preexisted in the parental Eb^{TGR} cell line because this would have resulted in a shift in the mortality curve.

Our findings demonstrate that the fusion of a nonmetastatic tumor with a bone marrow–derived normal host cell (macrophage) can lead to the generation of highly metastatic tumor variants. The relatively quick segregation of chromosomes after fusion leads to additional cellular heterogeneity, thus increasing even further the material from which the best-fitted variants could be selected by the host (14). That such a mechanism could indeed be an important parameter in tumor progression is suggested by recent findings from other tumor systems (15, 16). Not all somatic cell fusions, of course, may give rise to viable proliferating hybrids. We have observed with a different tumor line that not all hybridomas with normal cells will lead to generation of high metastatic variants (17). Intensive further studies along these lines are required to define which properties

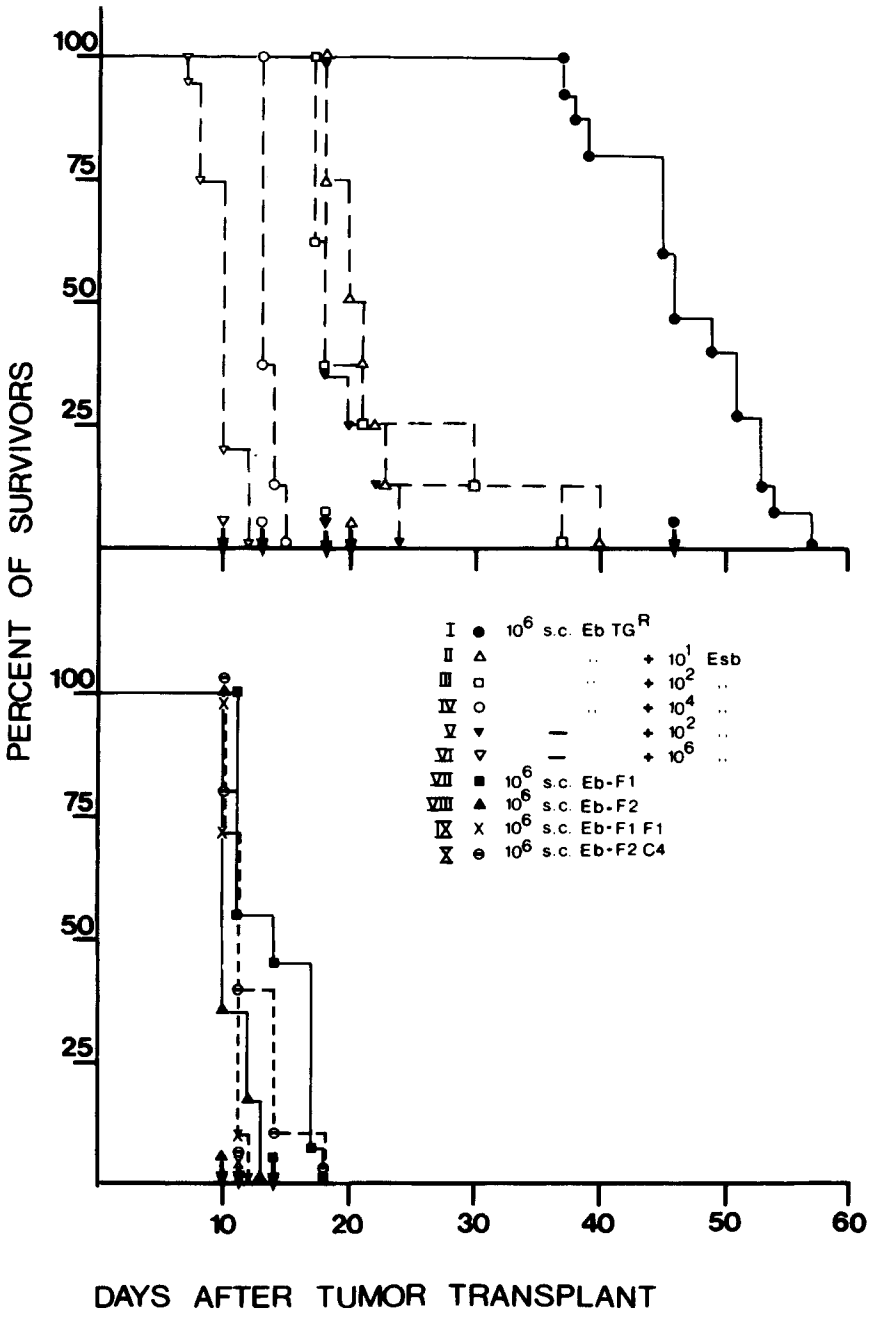


FIGURE 2. Survival curves of DBA/2J mice after subcutaneous inoculation of 10^6 cells of the high metastatic tumor macrophage hybridomas Eb-F1 and Eb-F2 (groups VII, VIII) (*bottom*) in comparison (*top*) to those of the low metastatic parental T lymphoma Eb^{TGR} (group I) and its spontaneous high metastatic in vivo variant ESb (group VI). Data from clones of Eb-F1 (Eb-F1F1), and Eb-F2 (Eb-F2C4) are included (groups IX and X). Groups II–IV show the shifts in the survival curves when small numbers of ESb cells were added to the Eb^{TGR} cells. Arrows point out the 50% survival time in each group.

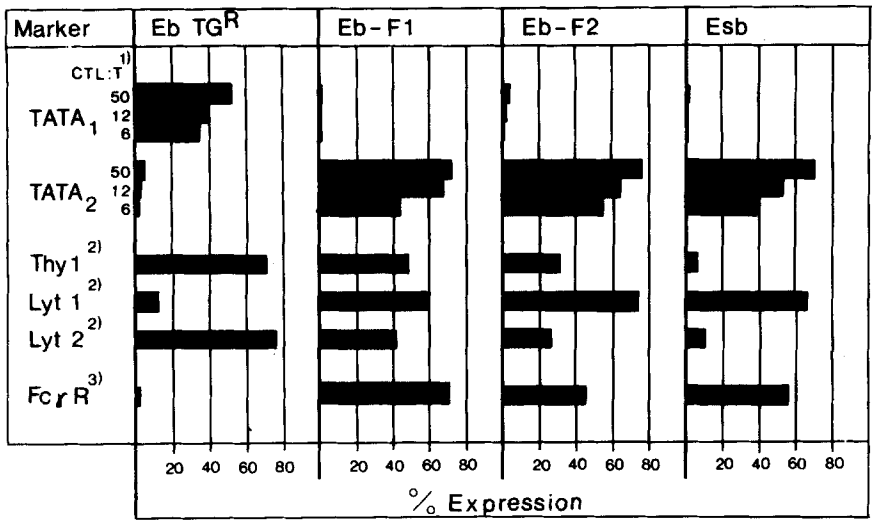


FIGURE 3. Cell surface marker expression by the high metastatic tumor macrophage hybridomas Eb-F1 and Eb-F2 in comparison with the low metastatic parental T lymphoma EbTG^R and the spontaneous high metastatic in vivo variant ESb. (*1*) Tumor-associated transplantation antigens type 1 ($TATA_1$) were typed using syngeneic tumor-specific cytotoxic T lymphocytes (CTL) raised against Eb type cells, and those of type 2 ($TATA_2$) by using CTL raised against ESb type cells (10, 14). Values are expressed as percent specific ^{51}Cr release from the respective target cells (*T*) after 4 h co-incubation of CTL and T at the indicated ratios. (*2*) Expression of lymphoid differentiation antigens was tested by monoclonal antibodies and cytofluorographic analysis as described (16). Columns indicate the percentage of specifically stained cells. (*3*) Percentage of EA rosette-forming cells tested as described (15). The original Eb lymphoma line is not shown because it had the same profile of marker expression as the Eb^{TGR} cells.

contributed by either tumor cells or host cells are important for metastatic potential and how the expression of these properties can be regulated and modulated.

Summary

A low metastatic, thioguanine-resistant murine T lymphoma line (Eb^{TGR}) was hybridized in vitro, with the help of polyethylene glycol, with syngeneic bone marrow–derived macrophages. Two HAT-resistant hybrid lines (Eb-F1 and Eb-F2) were obtained from independent fusion cultures. A cytogenetic analysis revealed that most of the macrophage chromosomes except No. 12 had segregated or become rearranged 60 d after fusion, a time at which the cell lines had become stabilized in culture. Syngeneic mice inoculated subcutaneously with the tumor macrophage hybrid lines developed, very quickly, visceral metastases and died after <2 wk, while those inoculated with the parental line lived for >6 wk and developed only localized, large primary tumors. The metastatic hybridomas expressed a similar tumor antigen as a spontaneous, in vivo derived, high metastatic variant (ESb) of the same tumor. This suggests that ESb cells might have arisen from a spontaneous fusion with a host macrophage.

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