CANINE TRACHEAL EPITHELIAL CELLS ARE MORE SENSITIVE THAN RAT TRACHEAL EPITHELIAL CELLS TO TRANSFORMING GROWTH FACTOR BETA INDUCED GROWTH INHIBITION

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Abstract — Transforming growth factor beta (TGFβ) markedly inhibited growth of canine tracheal epithelial (CTE) cells. Reduced responsiveness to TGFβ-induced growth inhibition accompanied neoplastic progression of these cells from primary to transformed to neoplastic. This was similar to the relationship between neoplastic progression and increased resistance to TGFβ-induced growth inhibition seen for rat tracheal epithelial (RTE) cells. The canine cells were more sensitive than rat cells to TGFβ-induced growth inhibition at all stages in the neoplastic process.

Transforming growth factor beta (TGFβ) is a multifunctional cellular regulator. The peptide is named for its capacity to produce anchorage-independent growth in the normal rat kidney fibroblast clone, NRK 49F, in the presence of TGFα or epidermal growth factor and serum-associated factors. TGFβ is the first described member of an entirely new class of homologous regulatory peptides.

TGFβ exists in at least 2 homodimeric forms, known as TGFβ1 and TGFβ2. A heterodimer of the subunits which make up TGFβ1 and TGFβ2 has also been described and is known as TGFβ1.2.[1] TGFβ1 is the major form of TGFβ present in porcine platelets and is the only form of TGFβ found in human platelets.[2]

The diverse actions of TGFβ include the following activities: (1) marked inhibition of cell growth, particularly in epithelial cells; (2) induction of anchorage-independent growth in normal fibroblasts; (3) inhibition of cellular secretion; (4) stimulation of cellular secretion; (5) stimulation of chemotaxis; and (6) matrix-dependent inhibition or stimulation of endothelial cell growth.[3] Of particular interest to us, TGFβ permits the growth of some, but not all, tumor-derived cell lines at concentrations that inhibit the growth of the corresponding primary cells. Using the rat tracheal epithelial (RTE) culture and transformation system as a model, we have recently found the development of resistance to TGFβ to be a consistent and late change in neoplastic progression (this report, pp. 381-387). We have also recently developed techniques for the growth and transformation of primary canine tracheal epithelial (CTE) cells in serum-free medium (1986-87 Annual Report, LMF-120, pp. 522-528). Because the cells of outbred and long-lived mammalian species, including man, are remarkably resistant to in vitro neoplastic transformation compared to rodent cells, we have compared the effect of TGFβ on normal, transformed, and tumor-derived rat and canine respiratory epithelial cells to determine if similar or different patterns of resistance to TGFβ occur during neoplastic progression.

MATERIALS AND METHODS

Active TGFβ1, of human platelet origin was purchased from R&D Systems, Inc., Minneapolis, MN and was 97% pure. RTE cells were harvested from the tracheas of 6-8 wk old F344 rats as previously described.[4] Preneoplastic RTE cells were the result of in vitro transformation of

primary RTE cells[5,6] and were shown to be nonneoplastic by inoculation into nude mice. Tumor-derived RTE cells were isolated from tumors appearing in nude mice inoculated with tumorigenic, transformed RTE cells. RTE cells were plated into serum-free medium developed for clonal proliferation of RTE cells[6] that we modified by lowering the BSA concentration to 0.05% and deleting the cholera toxin. For TGFβ testing, RTE cells were refed 2 days after plating with the same medium containing 0-300 pg/mL TGFβ1 (this report, pp. 381-387).

Primary CTE cells were harvested from tracheas of adult Beagle dogs born and raised at the Institute. These dogs had never been treated with chemical or physical carcinogens and were euthanized after they received latex beads as part of a pulmonary deposition study. We have previously described the technique for harvesting and transforming these cells (1986-87 Annual Report, LMF-120, pp. 522-528). At the passage used, the transformants were shown to be nonneoplastic by inoculation and/or tracheal repopulation in nude mice. A culture of tumor-derived cells was previously established from a radiation-induced lung tumor (CLEP4) isolated from a Beagle dog (1986-87 Annual Report, LMF-120, pp. 303-307). CTE cells were plated into serum-free medium developed for clonal proliferation of CTE cells (1986-87 Annual Report, LMF-120, pp. 522-528). For TGFβ testing, cells were refed 2 days after plating with CTE serum-free medium containing 0-300 pg/mL TGFβ1, but without cholera toxin.

The number of colonies of $\geq$ 30 cells were counted 10 days after plating. Percent colony-forming efficiency (% CFE) was calculated by dividing the number of colonies in each dish by the number of cells plated and multiplying by 100. Relative CFE was defined as the colony-forming efficiency of a treatment group relative to the colony-forming efficiency of the control (0 pg/mL) group, and was calculated by dividing the colony-forming efficiency in each treatment group by the colony-forming efficiency in the control (0 pg/mL) group.

To control for effects of media variation on TGFβ-induced growth inhibition, CTE cells derived from a single dog were run in a paired assay, with half of the cells treated identically to the RTE cells and half of the cells treated as routine CTE cells. A representative in vitro transformed CTE cell line, ACT 8, which grew well in RTE cell culture medium, was also examined for TGFβ sensitivity under conditions used for the RTE cells.

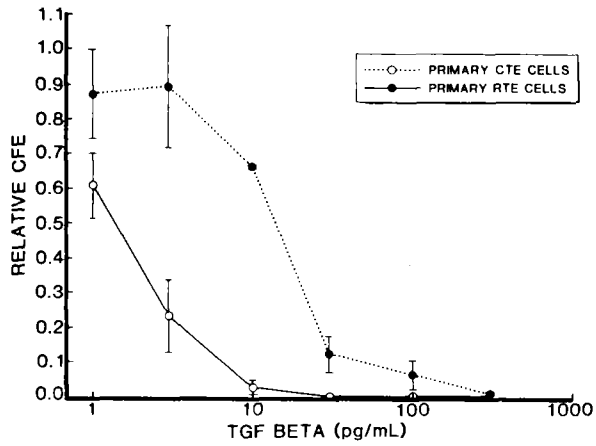
RESULTS

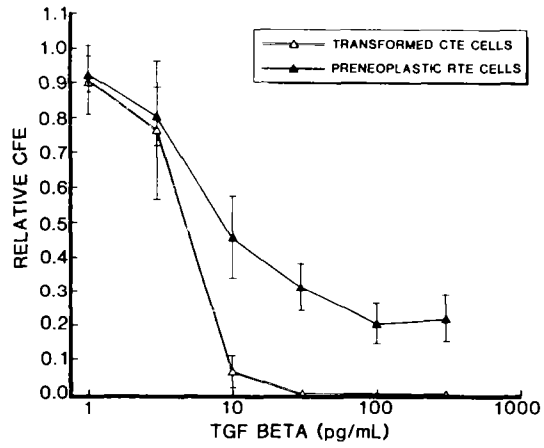
TGFβ inhibited the growth of primary CTE cells remarkably. This effect was concentration-dependent with a relative CFE of 0.5 between 1 and 3 pg TGFβ/mL, and with a relative CFE of 0 (no growth) at 30 pg/mL (Fig. 1A).

RTE cells were also highly sensitive to TGFβ-induced growth inhibition, but the growth inhibitory effects were observed at a log-dose higher concentration (Fig. 1A). A relative CFE of 0.5 occurred between 10 and 30 pg TGFβ/mL, and a relative CFE of 0 was seen at 300 pg/mL.

Transformed CTE cell lines were all consistently less sensitive to TGFβ than were primary CTE cells (Fig. 2) but were more sensitive than preneoplastic RTE cells (Fig. 1B). The TGFβ-induced growth inhibition of transformed CTE cells was virtually complete at concentrations of 10 to 30 pg/mL. In contrast, a canine lung tumor cell line, CLEP 4, was less sensitive to TGFβ than either the transformed or primary CTE cells (Fig. 2). CLEP 4 cells were, nevertheless, more sensitive to TGFβ than were tumor-derived RTE cells (Fig. 1C).

In a paired assay, treating primary CTE cells identically to the RTE cells only minimally reduced the effect of TGFβ (Fig. 3A). A relative CFE of 0.5 was still seen between 1 and 3 pg TGFβ/mL, and a relative CFE of 0 was seen at 30 pg/mL (although a single colony in 5 dishes was observed at concentrations of 100 and 300 pg TGFβ/mL). However, in another paired assay, plating and treating a representative in vitro transformed CTE cell line, ACT 8, in rat medium caused a





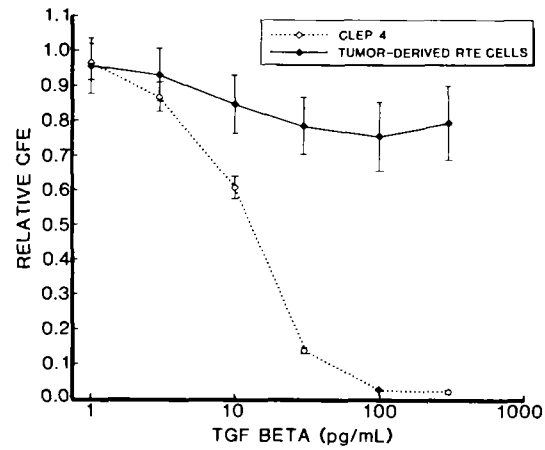


Figure 1. The comparative effects of TGFβ on canine and murine cells at different stages in neoplastic progression. Canine and murine cells were each plated in serum-free medium designed for clonal proliferation of those cells (see text). Medium was switched to medium containing TGFβ 2 days after plating. (A) Primary RTE and CTE response is the mean ± the standard error of the mean of two separate assays. (B) TGFβ sensitivity of transformed RTE and CTE cells is the mean for three different transformed CTE cell lines and seven different transformed RTE cell lines. (C) TGFβ sensitivity of tumor-derived canine cells is the mean ± the standard error of the mean of the lung tumor cell line, CLEP 4, in a single representative assay. (Variable cell plating densities had no effect of TGFβ sensitivity.) TGFβ sensitivity of tumor-derived RTE cells is the mean ± the standard error of the mean of five tumor-derived cell lines.

more noticeable reduction in the sensitivity to TGFβ at all concentrations $\geq$ 10 pg/mL relative to the TGFβ sensitivity of these cells in canine medium (Fig. 3B).

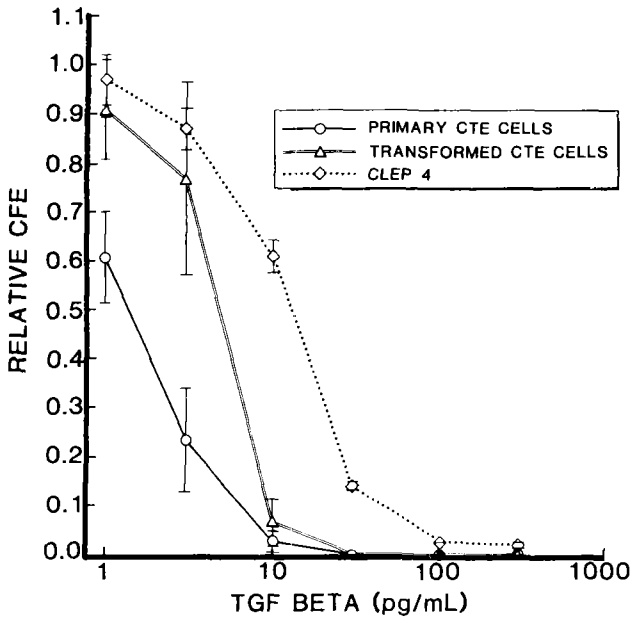


Figure 2. Comparative effect of TGFβ on canine respiratory epithelial cells at different stages in neoplastic progression. The data for primary CTE cells are the mean of two separate assays. The data for transformed CTE cells are the mean of assays on three separate transformed cell lines. The line for CLEP is the line for this lung tumor cell line in a single assay.

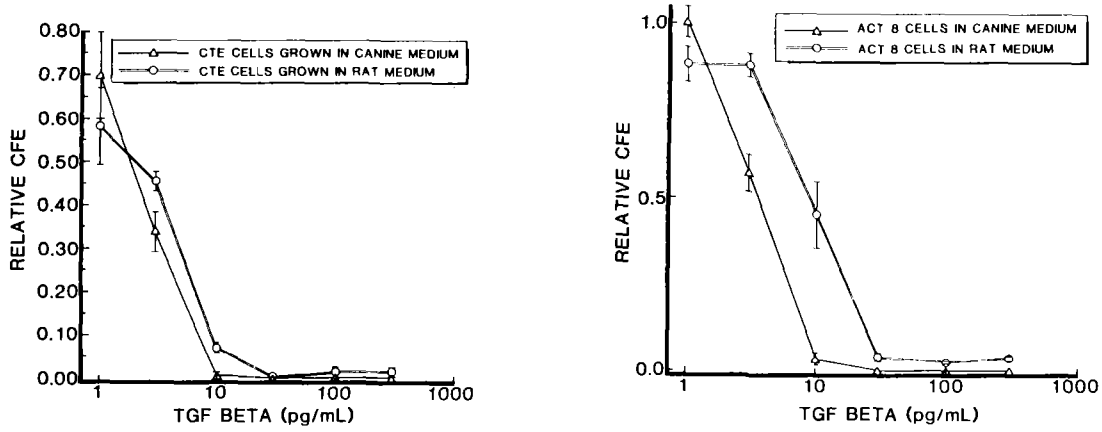


Figure 3. The effect of plating canine cells in canine or rat medium on responsiveness to TGFβ. These two experiments were performed as paired experiments with cells from the same source treated at the same time in the different media. (A) Primary CTE cells; (B) ACT 8, a transformed canine cell line which could grow in the rat medium and which had a response to TGFβ that was intermediate between that of the other two transformed CTE cell lines.

DISCUSSION

Numerous published reports document TGFβ-induced growth inhibition of a variety of epithelial cells.[3] No existing reports examine species differences in TGFβ susceptibility, although one report has examined TGFβ receptor number and affinity in a wide variety of species and tissue types.[7] In that report, the receptor number and receptor affinity are inversely related so that at physiological concentrations, an equal number of TGFβ molecules are bound to each cell. This relationship was consistent despite some differences in the culture conditions necessary to maintain the different cell lines.

In contrast to these results on TGFβ binding, our studies suggested that the effect of active TGFβ1 was not the same in respiratory cells from the Beagle dog and the F344/N rat. Primary CTE cells were approximately a log-dose more susceptible to TGFβ1-induced growth inhibition than the

primary RTE cells. The greater sensitivity of the canine vs. the murine respiratory epithelial cells was also observed for transformed and tumor-derived cells.

Differences in the media used for culturing could, theoretically, account for some of the differences observed. As previously described (1986-87 Annual Report, LMF-120, pp. 522-526), the canine medium contained lower concentrations of calcium (0.3 mM for the canine medium vs. 0.8 mM for the rat medium) and no hydrocortisone (concentration in the rat medium was 0.3 mM). Cholera toxin (1 nM) was used for plating, but not for treating, the CTE cells; cholera toxin was not present in either case for the RTE cells. However, CTE cells plated and treated under conditions used to treat the RTE cells were still more sensitive to TGFβ than were RTE cells, although increases in resistance were observed (Fig. 3). Changing plating conditions only minimally altered the TGFβ-induced growth inhibition of primary cells. Changing plating conditions of a representative transformed CTE cell line, ACT 8, shifted the TGFβ response curve approximately 1/2 log-dose to the right, but did not entirely explain the differences between species. Thus, some, but not all, of the different sensitivities to TGFβ can be attributed to the small differences in the serum-free media used for plating the cells from the two different species.

Several published reports are consistent with an essential role for TGFβ in most cell types: (1) cellular receptors for TGFβ are ubiquitous;[7] (2) TGFβ is a product of most normal as well as neoplastic cells; and, (3) TGFβ has remarkable interspecific sequence homology, with identical sequences for TGFβ1 of porcine, bovine, and human origin and only a single amino acid substitution between the murine and human polypeptides. Conversely, we have also shown that resistance to TGFβ-induced growth inhibition plays a role late in the neoplastic progression of RTE cells (this report, pp. 381-387). In the study reported here, we noted that the degree of sensitivity of tracheal epithelial cells to TGFβ-induced growth inhibition is species-dependent.

Our data also suggested that resistance to TGFβ occurred during neoplastic progression of respiratory epithelial cells from more than one species. However, at all stages in neoplastic progression, canine respiratory epithelial cells are more sensitive than rat respiratory epithelial cells to TGFβ-induced growth inhibition.

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