

Transforming growth factor α is trophic to pancreatic cancer in vivo

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Abstract

Pancreatic cancer cell lines overexpress epidermal growth factor (EGF) receptors and also have the capacity to produce transforming growth factor α (TGF α), the alternate agonist of the EGF receptor. The purpose of this study was to determine if TGF α had a trophic effect on the growth of pancreatic cancer in vivo. Syrian golden hamsters were inoculated with 50 000 H2T hamster ductal pancreatic cancer cells. The hamsters were then randomised to three equal groups: the groups received either saline (control), EGF, or TGF α , each by intraperitoneal injection, three times a day. Treatment continued for seven weeks, and each week the hamsters were weighed and tumour areas were measured. The hamsters were then killed, and the tumours were excised, weighed, and extracted for assay of DNA content as a measure of cellularity. From week four onwards both EGF and TGF α significantly stimulated tumour growth. Although tumour weights were not significantly different, tumour DNA content had nearly doubled after exposure to both EGF and TGF α . It is concluded that like EGF, TGF α can stimulate pancreatic cancer growth in vivo, and this may in part explain the aggressive nature of these cancers in clinical practice.

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Pancreatic carcinoma is now overtaking gastric cancer as the fourth leading cause of death from malignancy in the United Kingdom.¹ Most patients are dead within a year of the diagnosis and overall, less than 1% live more than five years.² The outlook for patients after the diagnosis of cancer of the exocrine pancreas is uniformly poor and suggests the existence of a mechanism(s) that may have a role in promoting tumour growth.

Korc and colleagues have shown that human ductal pancreatic cancer cell lines may overexpress the receptors for epidermal growth factor (EGF).³ The EGF receptor is a product of the *erb-B* oncogene.⁴ These cell lines can also produce the alternate agonist of the EGF receptor, transforming growth factor α (TGF α).⁵ Furthermore, it has been shown in several of these cell lines that this TGF α does not have to leave the cell to actively bind to transmembrane fragments of the EGF receptor and stimulate the cell.⁶ Therefore a potential growth stimulatory autocrine cycle may exist to explain the aggressive nature of these cancers.

The growth of established pancreatic cancers is promoted and pancreatic carcinogenesis is accelerated by EGF in animal models.^{7,8} The

purpose of this study was to examine the effect of TGF α on the growth of hamster ductal exocrine pancreatic cancer cells in vivo.

Methods

H2T hamster ductal pancreatic cancer cells (a gift from Dr Courtney Townsend Jr, Department of Surgery, University of Texas Medical Branch, Galveston, Texas, USA), which possess 5000 EGF receptors per cell, (R Beauchamp, Department of Surgery, University of Texas Medical Branch, Galveston, Texas, USA, personal communication), were grown (in Dulbecco's modified Eagle's medium (DMEM) containing 10% serum) for three days at which point they had achieved 80%–90% confluence. The cells were then treated with trypsin and suspended in a solution of DMEM at a concentration of 500 000 cells per ml. Eighteen six week old male Syrian golden hamsters (mean weight 70 g) were anaesthetised with intraperitoneal ketamine (100 mg/kg), their cheek pouches were everted, and 50 000 H2T cells (volume 0.1 ml) were inoculated under the mucosa of both cheek pouches. The hamsters were then randomised into three equal groups, housed throughout the study at 70°C in a 12 hour light/dark cycle, and given regular hamster food and tap water ad libitum. UKCCCR guidelines on the handling of animals for laboratory studies were rigorously adhered to throughout these investigations.

The three groups of hamsters received either saline, EGF (10 μ g/kg) (Peninsula, Warrington) or TGF α (10 μ g/kg) (Peninsula, Warrington), each by intraperitoneal injection three times a day throughout the seven week course of the study. The dose regimen was based on that previously cited for EGF as stimulating pancreatic carcinogenesis in hamsters.^{7,8} Each week the hamsters were weighed under ketamine anaesthesia, and the tumour areas calculated by measuring the bipedicular diameters with Vernier calipers. The hamsters were killed during week seven, the tumours were excised, weighed, and extracted for DNA estimation according to the method of Burton.⁹ Statistical comparison of tumour growth rate was by two way analysis of variance (time and treatment regimen) and tumour weight and DNA content by one way analysis of variance, *p* values <0.01 were considered significant for the two way analysis and *p* values <0.05 for the one way analysis.

Results

All three groups of hamsters increased their body weights at the same rate during the study and there were no differences in final mean body

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Tumour wet weights and DNA content in the three treatment regimes at death

	Control Mean (SEM)	EGF Mean (SEM)	TGF α Mean (SEM)
Wet tumour weight (g)	0.46 (0.05)	0.48 (0.07)	0.60 (0.08)
Tumour DNA content (mg)	100 (25)	260 (40)*	170 (30)*

* $p < 0.05$ greater than controls; EGF=epidermal growth factor; TGF α =transforming growth factor.

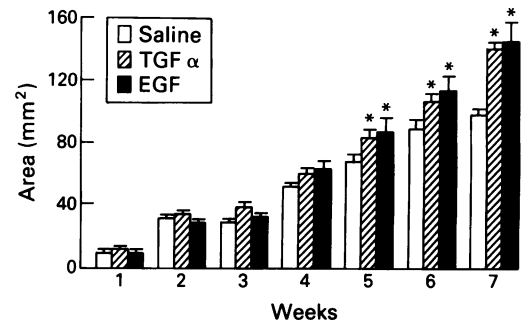
weights between the groups. All hamsters produced tumours. From week four until death both EGF and TGF α stimulated tumour growth at a significantly greater rate than that in the control group (Figure). Mean tumour doubling time was reduced from 18.9 days in the control tumours to 12.6 days in those hamsters receiving TGF α and halved to 9.1 days in the hamsters receiving EGF. At the time of killing most tumours had developed central ulcers with loss of tumour volume. Although tumour wet weights were not significantly different (Table), tumour DNA content was significantly higher in both treatment groups compared with the control group (Table).

Discussion

This study clearly shows that like EGF, TGF α will stimulate the growth of ductal exocrine pancreatic cancer in vivo. This is the first demonstration of a trophic effect for TGF α on a tumour in vivo. Moreover, the study also shows that TGF α has the capacity to act in an endocrine manner, being biologically effective at a site remote from the site of administration. Although it is not yet known if the H2T cell line expresses TGF α , these findings go some way to support the hypothesis proposed by Korc and others^{3,5,6} that TGF α may have a role as an autocrine growth stimulator in pancreatic cancer. If H2T cells are shown to produce endogenous EGF or TGF α , then our studies show an additional capacity to respond to further exposure to these agonists in vivo.

Complementary studies are now necessary to examine the effect of exogenously administered TGF α on the growth of the cell lines studied by the group of Korc *et al* when these cells are inoculated as xenografts into nude mice.

Clinically useful EGF antagonists do not exist. Schally and colleagues have proposed that the growth inhibitory effect of somatostatin and its long acting analogues may be via a tyrosine



The effects of EGF and TGF α on the growth of H2T hamster ductal pancreatic cancer cells in vivo. * $p < 0.01$, treatment groups greater than control.

phosphatase receptor. This in turn dephosphorylates the EGF receptor, which is a member of the tyrosine kinase receptor family.^{10,11} If this is the case, then there may be a clinical role for somatostatin analogues in the treatment of cancers that express both somatostatin and EGF receptors, regardless of their capacity to locally produce TGF α or EGF.

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