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RT-PCR DETECTION OF CYTOKINE TRANSCRIPTS IN A SERIES OF CULTURED HUMAN MENINGIOMAS

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SUMMARY

The expression of cytokine transcripts has been investigated in a series of cultured human meningiomas using reverse transcriptase linked polymerase chain reaction (RT-PCR), which allowed simultaneous analysis of a range of cytokines. The main histological subgroups of meningioma were investigated; these included transitional, fibroblastic, and syncytial as well as atypical meningiomas. Meningiomas from each of the different histological subgroups were subjected to a standard tissue culture regime. Total RNA was extracted from representative cultures and reverse-transcribed to yield cDNA. PCR was performed using oligonucleotide primers designed to detect interleukin (IL)-1α/β to IL-8, transforming growth factor (TGF)β_{1-3}, tumour necrosis factor (TNF)α/β, and interferon (IFN)γ. Transcripts for IL-3, IL-6, IL-8, and TGFβ_3 were detected in all cultures. Transcripts for the three isomers of TGFβ were expressed in the transitional and fibroblastic meningioma cells. TGFβ_2 and TGFβ_3 transcripts were expressed in the syncytial and TGFβ_1 and TGFβ_3 in the atypical meningioma cells. IL-1β transcripts were expressed in fibroblastic and atypical cultures and TNFβ transcripts were expressed in syncytial and transitional cultures only. Transcripts for IL-1α, IL-2, IL-4, IL-5, IL-7, TNFα, or IFNγ were not detected in any of the meningioma cultures. This investigation using cells cultured from a small number of tumours from each of the classic histological subtypes suggests that there is a distinct pattern of cytokine mRNA expression linked with histological classification.

KEY WORDS—meningioma; cytokine; histological subtypes; PCR

INTRODUCTION

Meningiomas constitute between 13 and 19 per cent of all primary brain tumours and 12 per cent of all tumours of the spine.[1] Whether intracranial or spinal, the meningiomas are most commonly detected in the fourth and fifth decades of life. They are typically benign tumours but the incidence of perineural growth, growth at inoperable sites, and recurrence complicates traditional surgical management. Development of an adjuvant therapy has been hindered by limited understanding of the aetiology and growth of these tumours.

According to the World Health Organization (WHO) classification of brain tumours, there are three major histological groups of meningioma.[1] The first group comprises the main categories of classic meningioma: syncytial, transitional, and fibroblastic, as well as variants of the subtypes. The second group includes the angioblastic meningiomas, which are also divided into two distinct variants, namely the haemangioblastic and haemangiopericytic. The third group is constituted by the malignant meningiomas.[2] The majority of tumours in this study belong to the group of classic meningiomas.

It has been shown that epidermal growth factor (EGF)[3,4] and fibroblastic growth factor (FGF) stimulate human meningioma cell proliferation *in vitro*. The presence of EGF, transforming growth factor β (TGFβ), and platelet-derived growth factor (PDGF) receptors has been documented and these results raise the possibility that complex stimulation by growth factors promotes meningioma formation and growth *in vivo*.[5–7]

It has become apparent that cytokines, in addition to functioning as immunoregulatory proteins, also serve as paracrine, autocrine, and endocrine factors which modulate an array of cell functions ranging from proliferation to differentiation.[8–13]

While several studies have been performed to elucidate the functions of cytokines in other neoplasms, their role in meningioma growth is still not understood. We have previously demonstrated that human meningiomas produce interleukin-6 (IL-6) and that the addition of exogenous IL-6 stimulates human meningioma cell proliferation.[14] We have also demonstrated the presence of IL-8 mRNA and the production of IL-8 by cultured human meningiomas (unpublished results). IL-8 is a potent inhibitor of meningioma cell proliferation. IL-6 and IL-8 production was also modulated by other cytokines, which was demonstrated when meningioma cells were treated with IL-1β with a subsequent increase in IL-6 production. IL-4 was a less potent stimulator of IL-6 production. Both IL-1β and IL-4 have been shown to decrease meningioma proliferation *in vitro*.[14] These results suggest that there is a complex network of cytokine interaction present in human meningiomas and that this network is important in the growth of these tumours.

This work prompted us to investigate the expression of cytokine transcripts in a series of cultured human meningiomas, using reverse transcriptase linked PCR (RT-PCR) which allowed the simultaneous analysis

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of a range of cytokine transcripts, in an attempt to identify the factors possibly involved in the growth and development of meningiomas.

MATERIALS AND METHODS

Culture of human meningioma tissue

Human meningioma tissue was kindly provided by neurological centres throughout the U.K. Freshly resected meningioma tissue was placed in growth medium (GM: RPMI containing 10 per cent fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5 μg/ml fungizone) (Gibco BRL, Paisley, U.K.) and transported to the laboratory. The tissue was divided into small pieces of 2–5 mm^3 and digested overnight in 1 mg/ml collagenase D (Boehringer Mannheim, Lewes, U.K.) at 37°C. After incubation, the dispersed tissue was washed three times with GM and the resultant cell suspension seeded in a 25 cm^2 tissue culture flask. Periodic media changes were carried out according to the growth rate of the cultured cells. The cells were passaged (P1) and grown on coverslips and then fixed in neutral-buffered formalin. At each passage the fixed cells were subjected to standard histopathological analysis with antibodies to vimentin, epithelial membrane antigen, S-100, and cytokeratin, to confirm that the cells retained the characteristics of precultured meningioma tissue.

Preparation of RNA, cDNA, and PCR analysis

RT-PCR was performed on cultures from human meningiomas rather than on the whole tissue, due to the delay in transporting tissue samples to the laboratory. This results in the RNA being degraded by RNases, making any cDNA synthesis difficult.

All reagents used for the following steps were of molecular biology grade. Total RNA was isolated from confluent cultures of 11 meningiomas of similar passage using the guanidium thiocyanate method[15] and stored under ethanol at − 70°C. Five micrograms of RNA was used as a template for cDNA synthesis in a 50 μl volume containing the following: 0·5 mM each of dATP, dCTP, dGTP, and dTTP; 2 μg of oligo dT (both Pharmacia Biotech, St Albans, U.K.); 20 U RNase inhibitor (Boehringer Mannheim, Lewes, U.K.); 10 mM dithiothreitol; 6 mM $MgCl_2$; 40 mM KCl; 50 mM Tris–Cl (pH 8·3); and 20 U/μg RNA of moloney murine leukaemia virus (MMLV) reverse transcriptase (Gibco BRL, Paisley, U.K.). Control tubes where dH_2O replaced RNA were also included. The reaction was incubated at 37°C for 1 h and terminated by freezing at − 20°C. The PCR reaction mixture was made up in a 50 μl volume and contained 2 units of Thermoprime DNA polymerase; PCR buffer (both Advanced Biotechnologies, London, U.K.); 0·5 μg of each oligonucleotide primer;[16] 200 μM each of dATP, dCTP, dGTP, and dTTP (Pharmacia Biotech, St Albans, U.K.); 1·5 mM $MgCl_2$; and 1 μl of cDNA. Control tubes with cDNA replaced by dH_2O were included. Cytokine transcripts were analysed in parallel in a thermal cycler (Hybaid omnigene) using the following cycle: a denaturation step of 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 45 s, and a primer extension step of 72°C for 3 min. The primers were designed to span introns so that mRNA signals could be distinguished from signals from contaminating genomic DNA.[16] In addition, the PCR products had been previously sequenced and restriction-digested to confirm their identity.[16] PCR products were analysed by agarose gel electrophoresis and visualized by ethidium bromide staining.

RESULTS

The histological classification and the anatomical locations of the 11 meningiomas studied are summarized in Table I. All meningiomas were independently classified according to the WHO classification by the consultant neuropathologist at the neurological centres where the meningioma samples were obtained.

Positive tumour vimentin and EMA staining was retained in all the meningioma cultures as in the corresponding tumour sections (unpublished results).

The cytokine profiles of syncytial, transitional, fibroblastic, and atypical meningioma cultures are summarized in Table II. It can be seen that each meningioma subtype studied has its own unique cytokine profile. Cell cultures from the syncytial subtypes of meningiomas expressed transcripts for IL-3, IL-6, IL-8, TGFβ_2, and TNFβ. Cells from the transitional cultures expressed

Table I—Histological classification and anatomical location of the cultured human meningiomas

Meningioma designation	Histological subtype	Anatomical location
M1	Fibroblastic	Posterior cranial fossa
M2	Fibroblastic	Left sphenoid wing
M3	Fibroblastic	Anterior cranial fossa
M4	Syncytial	Right sphenoid wing
M5	Syncytial	Not known
M6	Syncytial	Orbital
M7	Transitional	Right sphenoid wing
M8	Transitional	Anterior cranial fossa
M9	Transitional	Posterior cranial fossa
M10	Atypical	Right sphenoid wing
M11	Atypical	Not known

Table II—Profiles of cytokine mRNA detected in a series of cultured human meningiomas

PCR transcripts	*n*	Interleukins	TGF	TNF	IFN
Syncytial	3	IL-3, 6, 8	β_2, β_3	β	ND
Transitional	3	IL-3, 6, 8	β_1, β_2, β_3	β	ND
Fibroblastic	3	IL-1β, 3, 6, 8	β_1, β_2, β_3	ND	ND
Atypical	2	IL-1β, 3, 6, 8	β_1, β_3	ND	ND

ND=not detected.

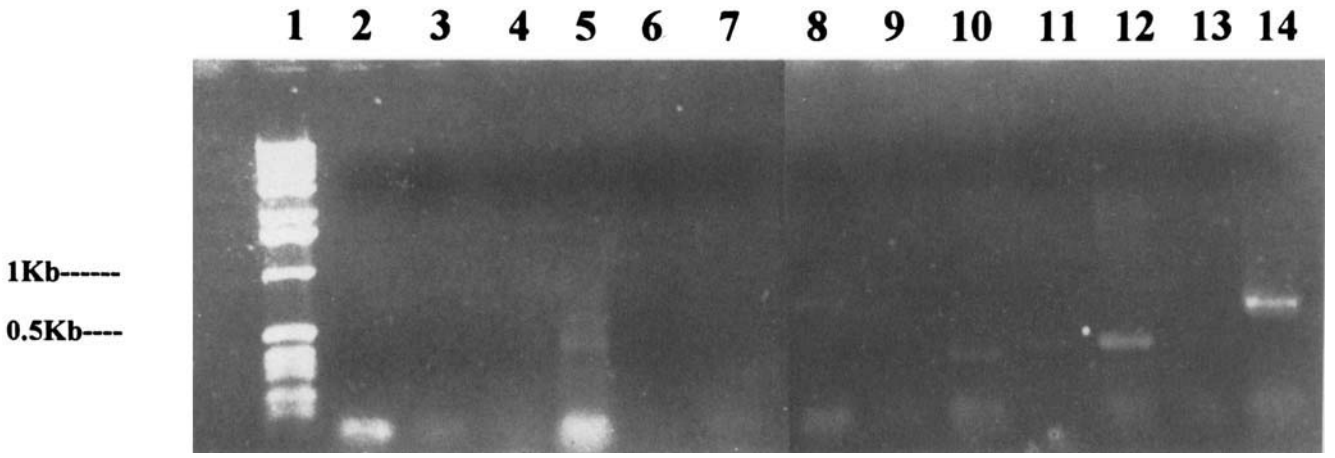

Fig. 1—Representative cytokine profile of a transitional human meningioma culture (M7) following RT-PCR. Lane 1=1 kb ladder (Gibco/BRL); lane 2=IL-1α; lane 3=IL-1β; lane 4=IL-2; lane 5=IL-3; lane 6=IL-4; lane 7=IL-5, lane 8=IL-6; lane 9=IL-7, lane 10=IL-8; lane 11=TGFβ_1; lane 12=TGFβ_2; lane 13=TGFβ_3; lane 14=β-actin

transcripts for IL-3, IL-6, IL-8, TGFβ_{1-3}, and TNFβ. Those from the fibroblastic subtypes expressed transcripts for IL-1β, IL-3, IL-6, IL-8 and TGFβ_{1-3}. Atypical meningioma cells expressed transcripts for IL-1β, IL-3, IL-6, IL-8, TGFβ_1, and TGFβ_3. Transcripts for IL-1α, IL-2, IL-4, IL-5, IL-7, TNFα, or IFNγ were not detected in any of the meningioma cell cultures tested.

A representative gel showing the PCR products from the transitional meningioma M7 is shown in Fig. 1. Transcripts for IL-3, IL-6, IL-8, TGFβ_{1-3}, and TNFβ (not shown) were detected.

Table III—A review of cytokine protein synthesis by cultured human meningiomas

Cytokine	Production
IL-1β	Not reported
IL-3	Not reported
IL-6	Detected in all meningioma cultures n=10 (ref. 15)
IL-8	Detected in all meningioma cultures n=9 (Boyle-Walsh, unpublished results)
TNFβ	Not reported
TGFβ_1	Detected in 2 out of 6 cultures (ref. 19)
TGFβ_2	Detected in 5 out of 6 cultures (ref. 19)
TGFβ_3	Detected in 5 out of 6 cultures (ref. 19)

DISCUSSION

Meningiomas are generally benign neoplasms of the brain and spinal cord and the factors involved in their aetiology and maintenance *in vivo* are still unclear. Previous work has shown that EGF and FGF stimulate meningioma growth *in vitro*.[4,5] Studies have also indicated that receptors for EGF, FGF, and PDGF have been located in meningiomas.[3,6,7] The production of IL-6 and TGFβ has been documented, as well as the receptors for TGFβ.[14,17–20] These observations raise the possibility that neoplasms of the meninges might synthesize other members of the cytokine family. In this study we have used RT-PCR to investigate the mRNA transcripts for cytokines expressed by cultured human meningiomas as an indication of possible cytokine production by these tumours. A review of cultured human meningioma cytokine production is shown in Table III.

Cytokine mRNA expression was studied in representatives of the three main categories of classic meningioma, syncytial, transitional, and fibroblastic, plus two atypical meningiomas. The cytokine mRNAs common to all meningiomas were those for IL-3, IL-6, IL-8, and TGFβ_3. TGFβ_1 and TGFβ_2 were found in a proportion of the meningiomas. TGF$\beta_{1,2\&3}$ mRNAs were expressed in the transitional and fibroblastic subtypes only; TGF$\beta_{2\&3}$ mRNAs in the syncytial only; and TGF$\beta_{1\&3}$ mRNAs in the atypical meningiomas.

The presence of mRNA for TGFβ was in agreement with previous reports which found transcripts for TGFβ in human meningiomas.[17] Johnson *et al.* detected transcripts for TGF$\beta_{1\&2}$ using Northern blot analysis. All three isomers of TGFβ were shown to be produced by cultured human meningiomas[17] (Table III). In our study, the expression of the different isomeric mRNAs was dependent on the histological subgroup.

IL-6 production has been documented in a seemingly endless variety of cells, including endothelial cells.[11,18,19] Our data show that IL-6 mRNA is expressed in all meningioma cultures. These results are supported by the fact that meningiomas secrete IL-6, as detected by bioassay and radioimmunoassay[14] (Table III). IL-6 causes growth stimulation in 60 per cent of meningiomas *in vitro*. IL-6 production can be increased when meningioma cells are pretreated with IL-1β or IL-4, but IL-1β and IL-4 alone cause a decrease in meningioma proliferation *in vitro*. Todo *et al.*[20] found cultured human meningioma IL-6 production to be stimulated by IL-1β, IL-4, and tumour necrosis factor α (TNFα), but addition of exogenous IL-6 (10–100 pg/ml) inhibited meningioma cell proliferation. These opposing cell proliferation results could be due to a concentration-dependent effect of IL-6, where at low concentrations IL-6 is inhibitory to cell proliferation, while at higher concentrations the

effect is reversed. These results suggest that a series of complex interactions may be important in the growth of these tumours.

Transcripts of IL-1β were detected in the atypical and fibroblastic human meningioma cultures, whereas transcripts for IL-1α were not detected in any of the cultures. IL-1β protein has not been detected in conditioned medium from any of the cultures studied (Table III). Analysis of the role of IL-1β in meningioma growth is complicated because this molecule can induce production of other cytokines, for example, IL-6.[14]

In this study, IL-3 was transcribed in all the meningiomas, irrespective of the subtype. It must of course be stressed that the detection of transcripts for a particular cytokine in the meningioma cultures is not a guarantee of protein production. The majority of previous studies have focused on the effects of IL-3 on haematopoietic cells, with few reporting effects on other cell lineages. IL-3 has been reported to regulate colonic carcinoma growth[21] but the significance of IL-3 in relation to meningioma growth has still to be elucidated.

IL-8 was also transcribed in all of the cultures studied, independent of the histological subtype. Previously we have shown that IL-8 is produced by cultured human meningioma cells and growth studies revealed that it is a potent inhibitor of meningioma cell proliferation *in vitro* (Boyle-Walsh, unpublished results) (Table III).

TNFβ mRNA was found in syncytial and transitional meningioma cultures, but not in the fibroblastic or atypical tumours. Although TNFβ promotes the growth of normal diploid fibroblasts and some tumour cells,[22] it is not yet clear whether TNFβ acts directly as a growth factor or whether it causes meningioma cells to produce another endogenous growth factor.

Messenger RNA for the cytokines IL-1α, IL-2, IL-4, IL-5, IL-7, TNFα, or IFNγ was not detected in any of the cultures, so it is possible that the absence of one or more of these cytokines may have a role in the development and growth of meningiomas. IFNγ has been shown to have anti-tumour activity and it has been used in clinical trials with some success.[23]

All meningioma samples retained immunohistochemical staining characteristics identical to those of the original tumour. It was feared that the cultures might have been contaminated and overgrown by highly selected, undesirable cell types, rendering them unsuitable representatives of the original meningiomas. Fibroblasts were ruled out on the basis that they have much higher cell densities and retain their characteristic spindle shape after repeated passages. It was unlikely that our cultures were contaminated by macrophages or endothelial cells, which do not survive in monolayer culture beyond the early passages without attention to their special metabolic requirements.[24]

Cytokines normally exert their diverse biological activities within the context of a cytokine network. Cytokine interactions play pivotal roles in many normal and pathological events, including the generation of immune responses, remodelling of tissues, and neoplastic transformation of cells. An imbalance in the production and/or action of cytokines within the network provides the basis for generating various pathological processes, such as neoplasia, which can be ameliorated by interfering in the production of cytokines using drugs and by intervening in the interactions of cytokines with their receptors through the use of antibodies or antagonists.

The findings presented here suggest that cultured human meningioma cells do in fact express mRNA for a series of cytokines and that the profile of expression corresponds to the histological subtype. The extent and pattern of expression of this array of cytokines in cultured human meningiomas have not previously been reported.

The data reported here may help to define patterns of cytokine genes which are transcribed in meningiomas of differing histological subtypes. This sensitive method of RT-PCR is an efficient way of detecting cytokine mRNA. The results can then be used in targeting the measurement of these cytokines. Their production and interaction may therefore be defined, which may help in isolating the important common factors in meningioma aetiology and growth.

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