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The effect of extracellular substance components on the proliferation and expression of hormones in cultured cells of medullary thyroid carcinomas

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The effects were examined of selected extracellular medium (ECM) components on the proliferation of medullary thyroid carcinoma cells and on the production of calcitonin and CGRP. Human TT cells and rat rMTC cells were cultured for 24, 48 and 72 hours on glass coated with type I collagen, fibronectin or poly-D-lysine. More pronounced proliferation was demonstrated by TT cells grown on poly-D-lysine or collagen in comparison with the control and less pronounced proliferation was typical of cells grown on fibronectin. On the other hand, rMTC cells were more markedly manifest on any ECM substrates than that on glass. Alterations in the proliferation were paralleled by changes in the expression of CT and CGRP.

Key words: medullary thyroid carcinoma, cell culture, extracellular matrix, calcitonin, CGRP

INTRODUCTION

Components of the extracellular matrix (ECM) affect the proliferation and function of normal and neoplastic cells [1]. The effects of the ECM carry a significant cognitive and practical significance, possibly explaining several developmental processes, the proliferation of tumour cells and the development of metastases. Such effects have been demonstrated in cases of multiple cultured tumour cells, including endocrine tumour cells [2, 3]. ECM components which have to be taken into account include laminins, collagen, fibronectin or synthetic substrates such as poly-D-lysine, representing a cationic polypeptide. Apart from being growth factors, ECM components are considered to be the main control elements of cellular proliferation, acting though surface receptors, termed "integrins". They mobilise intracellular mechanisms, which control mainly the G1 phase of the cell cycle and mechanisms linked to gene expression control [3].

In this study we decided to examine the effects of selected ECM components on the proliferation of cells of thyroid medullary carcinoma as well as on the production of calcitonin and CGRP.

MATERIAL AND METHODS

Medullary thyroid carcinoma cells (human TT cells and rat rMTC cells) were cultured for 24, 48 and 72 hours on a clean glass or glass coated with type I collagen, fibronectin or poly-D-lysine. Cell numbers were scored using a haemocytometer. Cells cultured for 72 h were stained immunocytochemically to de-

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tect the presence of calcitonin (CT) and CGRP and the reaction product was quantitated by measuring optical density [5, 6]. Additionally, in cells cultured for 72 h the presence of $\alpha2\beta1$, $\alpha4\beta1$, $\alpha5\beta1$ and $\alpha6$ integrins was detected immunocytochemically.

RESULTS AND DISCUSSION

The proliferation of rMTC cells was always more pronounced than that of TT cells. As compared to the control, stronger proliferation was demonstrated by TT cells growing on poly-D-lysine and collagen and less pronounced proliferation by cells grown on collage, with the proliferation exhibited by those cultured on fibronectin yet less pronounced (Fig. 1). On the other hand, rMTC cells demonstrated more pronounced proliferation on any ECM medium than on glass (Fig. 2). The differences were evident after 72 h. Changes in proliferation rates were accompanied by proportional alterations in the expression of CT and CGRP, which was particularly evident in cultures on collagen (Table 1). However, in each case the quantitative relation between the two hormones did not change. rMTC cells produced similar amounts of CT and CGRP but in TT cells CGRP production was almost twice as high as that of CT.

Of the membrane proteins examined, only the presence of $\alpha6$ integrin was demonstrated on the surface of TT cells (Fig. 3). Cells grew most rapidly On the sublayer of collagen and manifested the presence of long projections, best seen in rMTC cells (Fig. 4, 5). Shorter extensions were seen in cultures on fibronectin, while in cultures on glass or poly-L-lysine the projections were almost absent (Fig. 6–8).

The results indicate that the components of ECM tested affect proliferation and hormone expression from the calcitonin gene in the cells of thyroid medullary carcinoma. However, the rate of CT/CGRP expression remains unaltered, which indicates that ECM components do not affect the alternative splicing of the calcitonin gene transcript. In similar studies in cells grown on laminin inhibited proliferation of TT cells and CA-77 cells was detected, accompanied by a variable effect on the expression of the calcitonin gene [4]. The inhibitory effect of laminin on the proliferation of the cells may be explained by its presence in the basal membrane, which restricts cell growth. In our previous studies we demonstrated that the proliferation and expression of the calcitonin gene in TT cells is also affected by thyroid follicular cells [6].

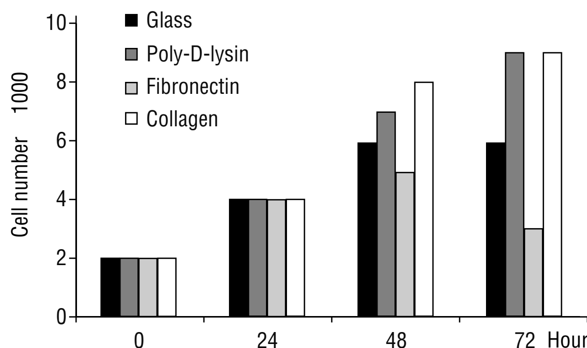

Figure 1. Effect of ECM components on the proliferation of TT cells up to 72nd hour.

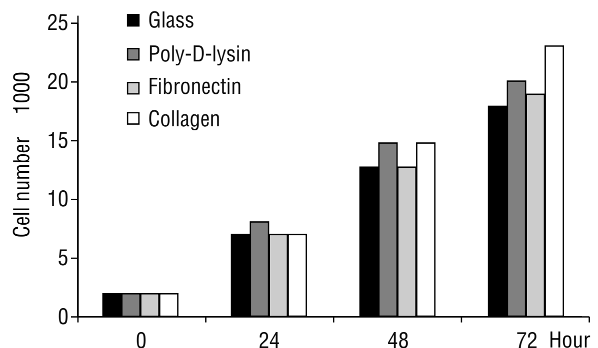

Figure 2. Effect of ECM components on the proliferation of rMTC cells up to 72nd hour.

Table 1. Intensity of CT and CGRP expression in TT and rMTC cells after 72 h of culture on various substrates

Substrate	TT cells			rMTC cells		
	CT	CGRP	CGRP/CT	CT	CGRP	CGRP/CT
Control (glass)	19.8 ± 3.8	34.8 ± 5.6	1.76 ± 0.21	13.8 ± 2.9	14.3 ± 3.2	1.04 ± 0.03
Poly-D-lysine	20.1 ± 4.2	32.8 ± 4.1	1.63 ± 0.14	15.1 ± 4.3	13.7 ± 2.6	0.91 ± 0.04
Fibronectin	16.6 ± 1.9	28.5 ± 3.9	1.72 ± 0.12	12.2 ± 2.4	13.1 ± 2.7	1.07 ± 0.04
Collagen type I	21.2 ± 3.7	36.5 ± 5.5	1.72 ± 0.24	15.3 ± 3.9	15.8 ± 4.3	1.03 ± 0.07

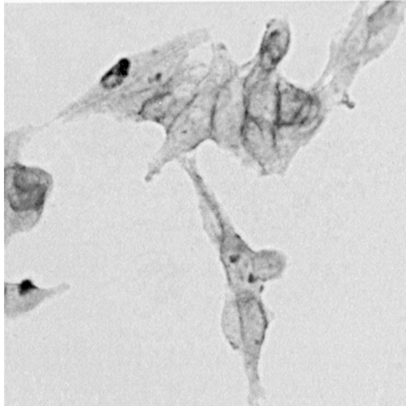

Figure 3. Immunocytochemical localisation of $\alpha 6$ integrin on the surface of TT cells cultured for 72 h on glass; 300 ×.

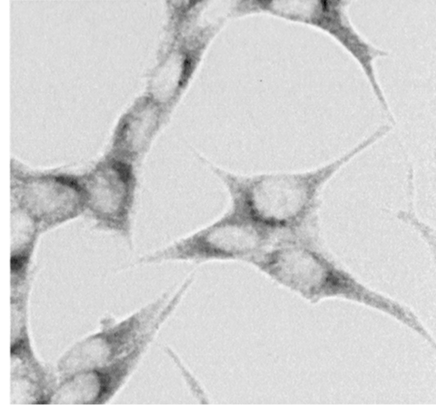

Figure 4. TT cells cultured for 72 h on collagen. Immunocytochemical reaction to detect CT; 600 ×.

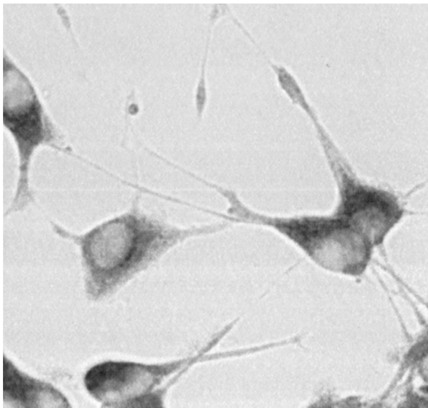

Figure 5. rMTC cells cultured for 72 h on collagen. Immunocytochemical reaction to detect CGRP; 450 ×.

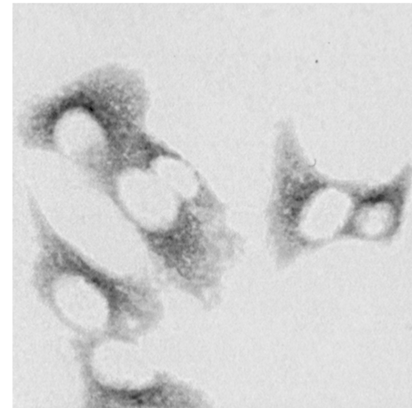

Figure 6. Immunocytochemical reaction to detect CT in TT cells cultured for 72 h on glass; 600 ×.

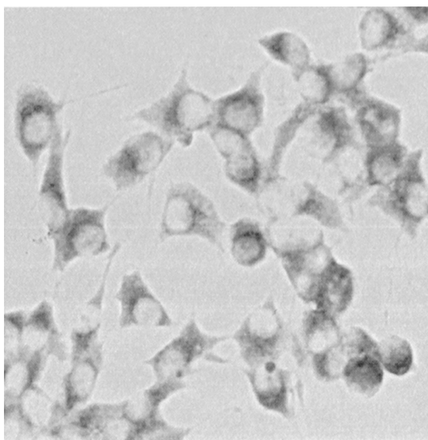

Figure 7. rMTC cells cultured for 72 h on fibronectin. Immunocytochemical reaction to detect CT; 300 ×.

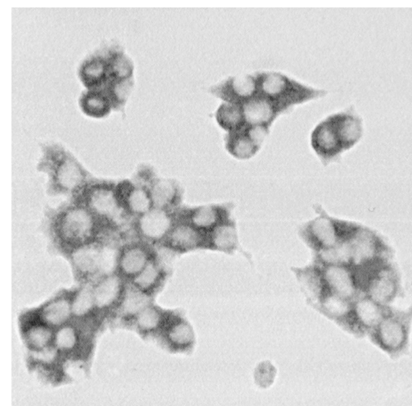

Figure 8. rMTC cells cultured for 72 h on glass. Immunocytochemical reaction to detect CGRP; 300 ×.

REFERENCES

1. Danen EH, Yamada KM (2001) Fibronectin, integrins, and growth control. J Cell Physiol, 189: 1–13.
2. Huet C,Pisselet C, Mandon-Pépin D, Monget P, Monniaux D (2001) Extracellular matrix regulates ovine granulosa cell survival, proliferation and steroidogenesis: relationships between cell shape and function. J Endocrinol, 169: 347–360.
3. Kuchenbauer F, Hopfner U, Stalla J, Arzt E, Stalla GK, Paez-Pereda M (2001) Extracellular matrix components regulate ACTH production and proliferation in corticotroph tumor cells. Mol Cell Endocrinol, 175, 141–148.
4. Lekmine F, Lausson S, Pidoux E, Segond N, Roos B, Treilhou-Lahille F, Jeanne A (1999) Influence of laminin substratum on cell proliferation and CALC I gene expression in medullary thyroid carcinoma C cell lines. Mol Cell Endocrinol, 157: 181–189.
5. Zabel M, Dietel M, Gebarowska E, Michael R (1999) Effect of follicular cells on calcitonin gene expression in thyroid parafollicular cells in cell culture. Histochem J, 31: 175–180.
6. Zabel M, Seidel J, Kaczmarek A, Surdyk-Zasada J, Grzeszkowiak J, Górny A (1994) Hybridocytochemical and immuno-ultrastructural study of calcitonin gene expression in cultured medullary carcinoma cells. Histochemistry, 102: 323–327.