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Downregulation of the *RECK*-Tumor and Metastasis Suppressor Gene in Glioma Invasiveness

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Abstract Invasive behavior is the pathological hallmark of malignant gliomas, being responsible for the failure of surgery, radiation, and chemotherapy. Matrix metalloproteinases (MMPs) are essential for proper ECM remodeling and invasion. The tumor and metastasis suppressor *RECK* protein regulates at least three members of the MMPs family: MMP-2, MMP-9, and MT1-MMP. In order to mimic the in vivo invasion process, A172 and T98G, respectively, non-invasive and invasive human glioblastoma cell lines, were cultured onto uncoated (control) or type I collagen gel-coated surface, and maintained for up to 7 days to allow establishment of the invasive process. We show that the collagen substrate causes decreased growth rates and morphological alterations correlated with the invasive phenotype. Electronic transmission microscopy of T98G cells revealed membrane invaginations resembling podosomes, which are typically found in cells in the process of crossing tissue boundaries, since they constitute sites of ECM degradation. Real time PCR revealed higher *RECK* mRNA expression in A172 cells, when compared to T98G cells and, also, in samples obtained from cultures where the invasive process was fully established. Interestingly, the collagen substrate increases *RECK* expression in A172 cells and the same tendency is displayed by T98G cells. MMPs-2 and -9 displayed higher levels of expression and activity in T98G cells, and their activities are also upregulated by collagen. Therefore, we suggest that: (1) *RECK* downregulation is critical for the invasiveness process displayed by T98G cells; (2) type 1 collagen could be employed to modulate *RECK* expression in glioblastoma cell lines. Since a positive correlation between *RECK* expression and patients survival has been noted in several types of tumors, our results may contribute to elucidate the complex mechanisms of malignant gliomas invasiveness. J. Cell. Biochem. 99: 156–167, 2006.

Key words: *RECK*; MMPs; collagen; glioma

Malignant gliomas (World Health Organization grade III or IV) are the most common primary brain tumor [Behin et al., 2003; Chang et al., 2005]. One of the most important hallmarks of malignant gliomas is their invasive behavior, rendering them a particularly challenging clinical problem [Demuth and Berens, 2004]. The invasive behavior is a critical prognostic factor for primary brain tumors since invasive tumors are almost invariably fatal, recurring near the resection margin in almost all cases. This fatal category of primary brain tumors includes glioblastomas, anaplasic astrocytomas, low-grade astrocytomas, and oligodendromas [Kleihues et al., 2002; Hunter et al., 2003]. An important group of enzymes, the matrix metalloproteinases (MMPs), has been related to the invasion process in almost all types of tumors, including gliomas.

In the last few years, MMPs have been pointed as promising targets for cancer therapy,

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on the basis of their massive upregulation in malignant tissues and their singular ability to degrade all components of the extracellular matrix (ECM) [Coussens et al., 2002]. The enzymes which degrade type IV collagen, namely: MMP-2 and MMP-9, also known as gelatinases A and B due to their characteristic activity, have been closely linked with the ability to degrade basement membranes and, hence, with the biological and malignant properties associated with this capacity [Fassina et al., 2000]. ECM degradation by MMPs not only facilitates tumor invasion, but, also, modifies tumor cell behavior, leading to cancer progression [Itoh and Nagase, 2002; Rao, 2003; Takino et al., 2004]. MMPs are able to regulate a wide range of cellular events, such as ECM remodeling and cell behavior, cell surface proteolysis and intracellular signal transduction, regulation of paracrine signals and generation and inactivation of bioactive molecules [Sternlicht and Werb, 2001]. These correlations between MMPs and cancer progression led to the development of synthetic metalloproteinases inhibitors (MPIs) for human clinical trials, however, the results of these trials were disappointing, with no evidence of therapeutic benefits for patients [Coussens et al., 2002; Egeblad and Werb, 2002; Rao, 2003]. Side effects were associated to prolonged treatment with MPIs. Also, in some cases, MMP inhibition stimulated disease progression resulting in more aggressive tumors [Coussens et al., 2002]. Since MMPs play an important role in tumor progression and the clinical trials with their synthetic inhibitors failed to prevent cancer development, their natural inhibitors deserve special attention. Several distinct classes of proteins have been found to inhibit MMPs activity, including tissue inhibitors of metalloproteinases (TIMPs) as well as a new class of membrane protease inhibitors represented by reversion-inducing-cysteine-rich protein with Kazal Motifs (*RECK*). The *RECK* gene is unique in that it encodes a membrane-anchored glycoprotein with serine protease inhibitor-like domains and is associated with the cell membrane through a COOH-terminal glycosylphosphatidylinositol modification [Takahashi et al., 1998]. *RECK* was described as a tumor and metastasis suppressor gene, as well as an angiogenesis suppressor and regulator of ECM integrity [Takahashi et al., 1998; Sasahara et al., 2002]. The *RECK* product negatively regulates at least three different MMPs, namely: MMP-2, MMP-9, and MT1-MMP [Oh et al., 2001]. In particular, MMP-2 [Brooks et al., 1996] and MMP-9 [Rao et al., 1993] are closely related to glioma invasiveness, rendering them a good target for glioma therapy.

Several clinical studies, with different kinds of tumors, describe *RECK* as a potential prognostic indicator, with its presence correlating with increased patient survival [Furumoto et al., 2001; Masui et al., 2003; Span et al., 2003; Takenaka et al., 2004; Takeuchi et al., 2004; van Lent et al., 2005].

In order to evaluate the involvement of *RECK* and MMPs in the glioma invasive process, two glioblastoma cell lines were chosen, namely: the non-invasive A172, and T98G, which displays an invasive behavior. Collagen gel was used as a model for the invasion process. Our results indicate that *RECK*, as a natural MMPs inhibitor, is likely to be a good target for cancer therapy.

EXPERIMENTAL PROCEDURES

Cell Cultures

A172 and T98G cell lines were purchased from ATCC (Manassas, VA). These cells were grown in Dulbecco's modified Eagle's minimum essential (DMEM) medium supplemented with 10% fetal bovine serum (FBS), 50 U/ml penicillin and 50 μg/ml streptomycin and maintained at 37°C under 5% CO_2 atmosphere. Type 1 collagen gel, extracted from rat-tail tendons, was prepared as previously described [Maria and Wada, 1997] with minor modifications. For growth curves, 1×10^4 cells of both A172 and T98G cell lines were plated in 24-wells plates onto either plastic or collagen (175 μl/well, 2.5 mg/ml). Triplicates were collected on days 1, 3, 5, and 7, using trypsin, fixed in 3.7% formaldehyde and counted using the Neubauer chamber. Graphs were generated using Microsoft Excel.

Electronic Transmission Microscopy

For light microscopy, cultures maintained onto the collagen gel for 7 days were immediately fixed in Karnovsky fixative (0.1 M Sörensën phosphate buffer, pH 7.2, containing 5% paraformaldehyde and 2.5% glutaraldehyde). Fixed samples were dehydrated in a grade series of ethanol and embedded in paraffin (Histosec, Merck) or glycol methacrylate

resin (Leica historesin embedding kit). Sections (3 μm thickness) were stained with hematoxylin–eosin [Behmer et al., 1976] for the general studies.

These sections were observed under either a Zeiss Jenaval or an Olympus light microscope. Tissue fragments were fixed by immersion into 3% glutaraldehyde plus 0.25% tannic acid solution in Millonig's buffer, pH 7.3, containing 0.54% glucose, during 24 h [Cotta-Pereira et al., 1976]. After washing with the same buffer, the material was post-fixed with 1% osmium tetroxide for 1 h, washed again, dehydrated in graded acetone series, and embedded in Araldite. Ultrathin silver sections were cut using a diamond knife and contrasted with 2% alcoholic uranyl acetate [Watson, 1958] and then with 2% lead citrate in 1 N sodium hydroxide solution for 10 min [Venable and Coggeshall, 1965]. The grids were examined under a Leo-906 transmission electron microscope operating at 80 kV.

RNA Extraction and Reverse Transcription

Total RNA was prepared from A172 and T98G cell lines cultured either on uncoated plastic (control) or onto type 1 collagen gel for 7 days, using the cesium chloride method as previously described [Chirgwin et al., 1979]. Briefly, cells were washed three times with PBS and lysed directly in the plates by adding lysis buffer (4 M guanidine isothiocianate, 25 mM sodium citrate, 7 μl of β-mercaptoethanol 4.4 M per ml). The lysate was centrifuged, at 37,000 rpm, 16–18 h, the supernatant was discarded and the RNA, sedimented at the bottom of the tube, was ressuspended in sterile milli-Q water. RNA concentration was determined by spectrophotometry. A total of 1 μg of total RNA, previously treated with DNase I (Invitrogen), was reverse transcribed using 250 ng of oligo-dT primers (Amersham), 50 ng of random primers and Superscript II reverse transcriptase (Life Technologies, Paisley, UK). The cDNA was then stored at −20°C until the PCR reactions were carried out.

Quantitative Real-Time PCR

For PCR reactions, specific primers were used. Primers for MMP-2, -9, MT1-MMP, and TIMP-2, designed by R.C.S. Figueira and M.C. Sogayar, using the Primer3 software, and synthesized by Invitrogen (Carlsbad, CA), will be published elsewhere. Primers sequences for *RECK* and tubulin, synthesized by IDT, Integrated DNA Technologies, Inc. (Coralville, IA) are shown in the 5′–3′ orientation: hRECK FTGCAAGCAGGCATCTTCAAA; RACCGAGCCCATTTCATTTCTG.; hTubulin FTCAACACCTTCTTCAGTGAAACG, RAGTGCCAGTGCGAACTTCATC.

Where possible, in order to prevent amplification of genomic DNA and to ensure that the PCR signal was generated from cDNA, the primers were generated from sequences within different exons, close to intron–exon boundaries. BLASTN searches [Altschul et al., 1990] were conducted for all primer sequences to ensure gene specificity. The tubulin gene was used as an endogenous control to normalize for differences in the amount of total RNA in each sample. PCR was carried out using Gene Amp 5700 Sequence Detection System (PE Applied Biosystems), with the SyBr Green Master mix (PE Applied Biosystems). Each PCR reaction contained 0.8 ng of reverse transcribed RNA, 6 μl of SyBr Green master mix and 600 nM of forward and reverse primers.

Gelatin Zymography

Gelatin-substrate gel electrophoresis was used to indirectly measure the levels of metalloproteinase activity in samples obtained from the A172 and T98G cell lines, as previously described, with minor modifications [Takahashi et al., 1998; Kato et al., 2002]. Cells were seeded onto plates coated with type 1 collagen gel or uncoated plastic (control) and maintained in DMEM containing 10% FBS. When cultures reached approximately 80% confluence, the medium was removed, and the cultures were washed three times with serum-free DMEM to remove residual FBS. Cells were then cultured for 48 h in DMEM containing 0.1% BSA. After 48 h, the culture medium was collected, and clarified by centrifuging twice at 800 rpm for 5 min. Protein concentration was measured using the Folin method [Lowry et al., 1951]; 25 μg of the supernatant of each sample was electrophoresed on a 0.8 g/ml gelatin, 10% acrylamide gel. The gel was washed twice with 2.5% Triton, for 15 min, at 37°C. The gel was then washed with reaction buffer (0.05 M TrisHCl, 5 mM $CaCl_2$, 5 μM $ZnCl_2$, pH = 8 and incubated for 16–17 h in the same buffer at 37°C. Gels were stained with Coomassie blue and de-stained. Gelatinolytic activity was visualized as clear white bands against a blue

background. EDTA 0.5 mM and 0.5 mM Ilomastat (GM6001, Chemicon, Temecula, CA) were used as negative controls of MMPs activities (data not shown).

Statistical Analysis

Data were expressed as the mean $\pm$ SD (standard deviation). Statistical analysis was carried out with the Graph Pad InStat software (version 3.01 for Windows 95, Graph Pad Software, San Diego, CA). One-way ANOVA with a multiple comparison test (Tukey test) was used for data analysis. Statistical significance was defined by a $P < 0.05$.

RESULTS

Growth Curves

Growth of A172 and T98G cells plated onto two different substrates is shown in Figure 1. The results show that: (i) the growth rate of T98G cells is higher than that of the noninvasive A172 cells, with doubling time of 22 and 37.5 h, respectively (ii) both cell lines display lower growth rates when cultured onto the collagen-coated surface (doubling time of 32 and 57.5 h, respectively). Also, both A172 and T98G cells underwent dramatic morphological changes when cultured onto the collagen gel (Fig. 2B,D) when compared to uncoated plastic (Fig. 2A,C), displaying plasma membrane alterations resembling the glial cells morphology (arrows).

Morphological Analysis by Optical and Electronic Transmission Microscopy

The histological analysis of A172 and T98G cells cultured onto collagen gels for 7 days revealed that A172 cells remain on the surface of the substrate (Fig. 3A,B), while T98G migrate to the interior of the gel (Fig. 3C,D). Several layers of cells (Fig. 3D) and a large number of mitosis were detected inside the collagen substrate of the T98G sections, illustrating the invasive nature of these cells.

Ultrastructural analysis of A172 cells showed a smooth surface of contact with the substrate, without protrusions or discrete invaginations, which are commonly related to cell invasion (Fig. 4A,B). On the other hand, ultrastructural analysis of T98G cells revealed a large number of protrusions of the plasma membrane (Fig. 5A,

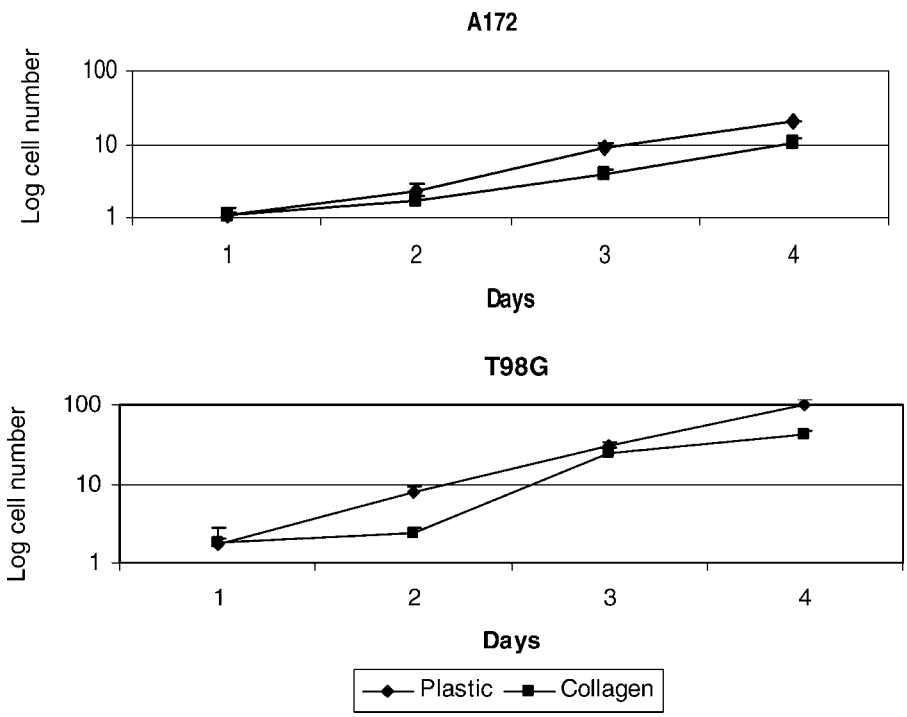

Fig. 1. Growth curves for A172 and T98G cell lines using two different substrates: uncoated plastic (control) and type 1collagen gel-coated wells. T98G cells, which are invasive, displayed higher growth rates than the noninvasive A172 (doubling time of 22 and 37.5 h, respectively). For both cell lines, collagen caused decreased cell growth (doubling time of 32 and 57.5 h, respectively for T98G and A172).

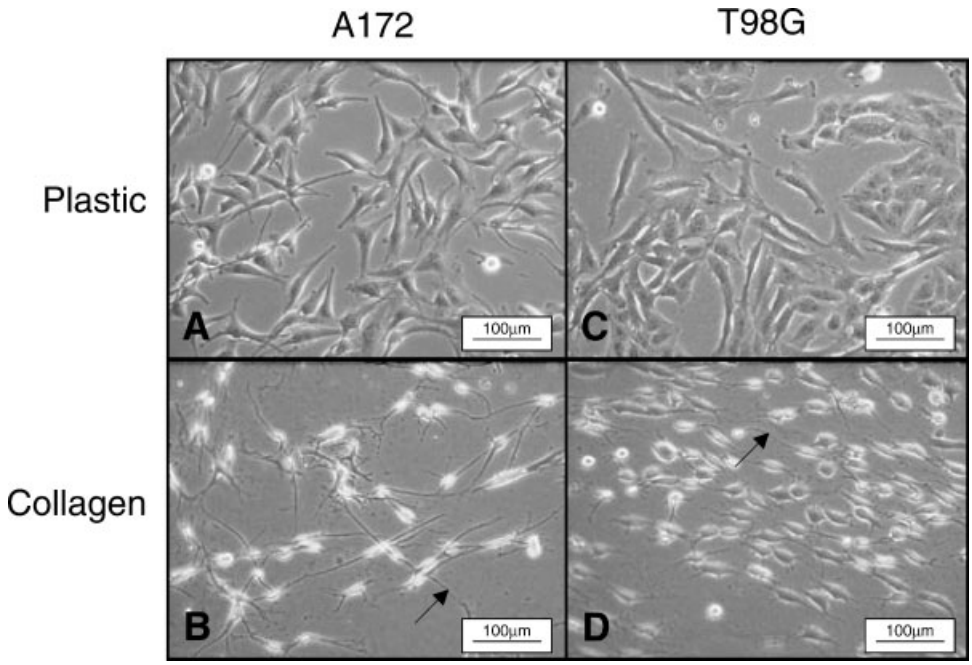

Fig. 2. Phase contrast microscopy of A172 (**A**, **B**) and T98G (**C**, **D**) glioma cells cultured onto uncoated plastic (A, C) or Type I collagen gel (B, D). Cells plated onto the collagen substrate depict alterations of plasma membrane, resembling glial morphology.

arrows), reorganization of the collagen fibers (Fig. 5B), substrate phagocytosis (Fig. 5C–C″) and regions of tubular podosome-like invaginations (Fig. 5B, upper arrow), described as sites of ECM degradation. T98G cells can also induce contraction of the collagen substrate, as observed in Figure 5B, where it is possible to observe collagen fibers in a new organizational arrangement nearby the invasion region (lower arrow). Taken together, these characteristics comprise the invasive behavior of the T98G cell line.

Imbalance of *RECK*, TIMP-2, MMPs-2 and -9, and MT1-MMP Expression Is Associated With the Invasiveness Process

To determine whether the *RECK* gene and the matrix metalloproteinases, which are

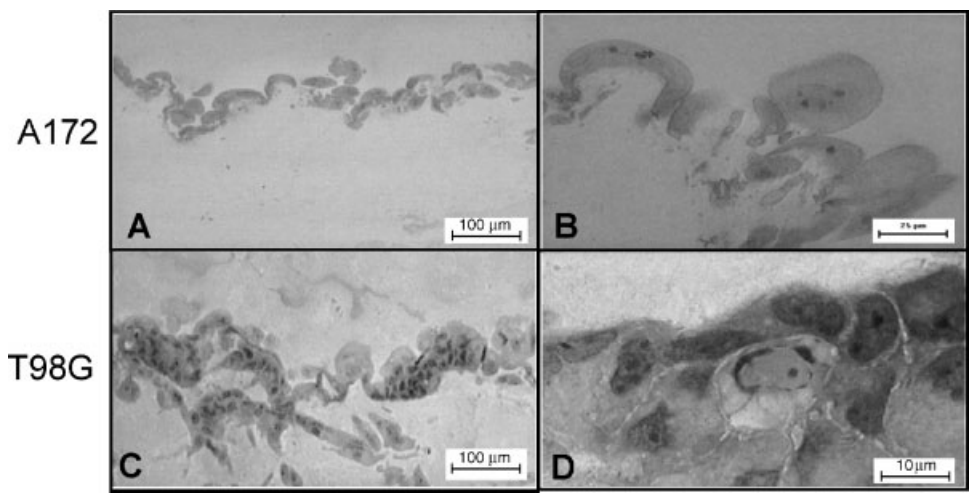

Fig. 3. Light microscopy showing A172 cells (**A**, **B**) remaining on the substrate surface and T98G cells invading the type 1 collagen gel (**C**, **D**). Sections were obtained after 7 days culture.

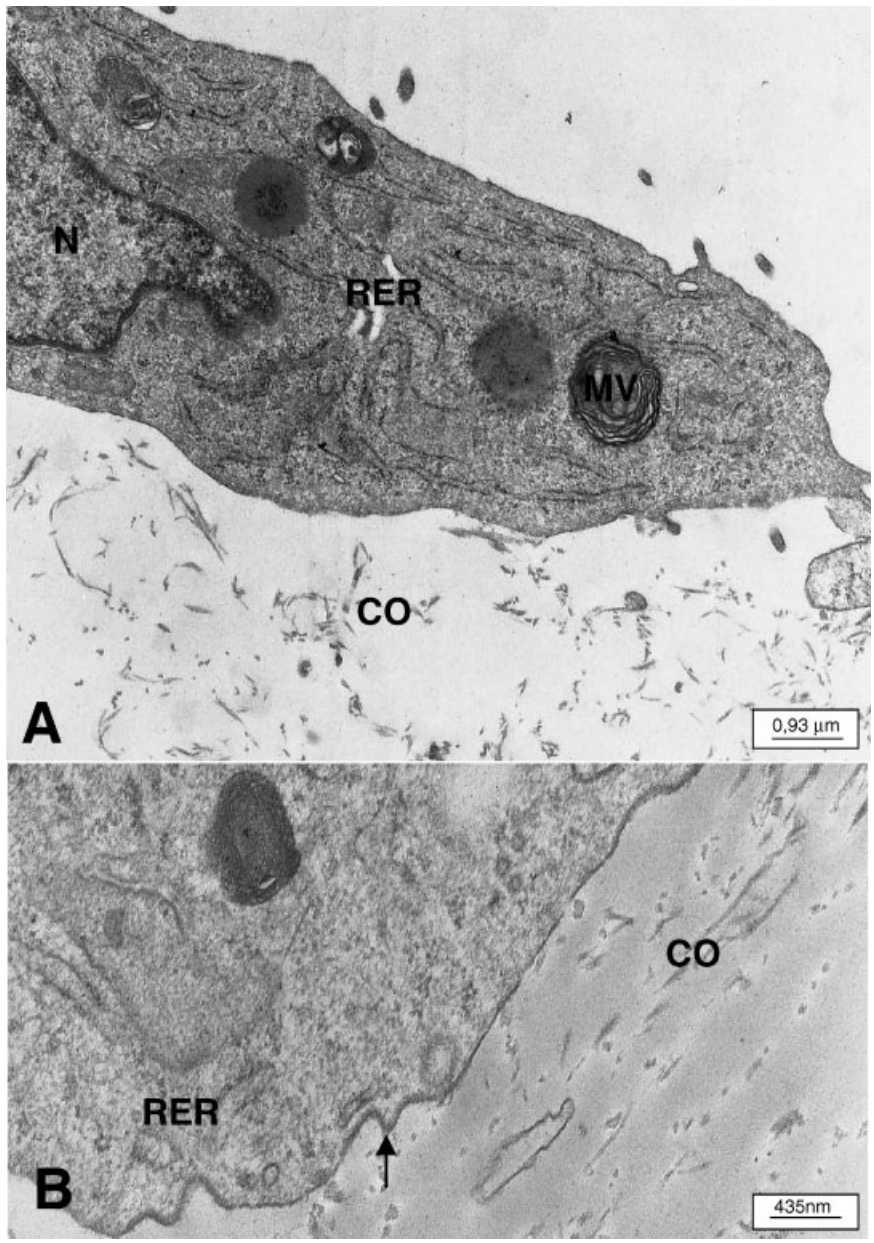

Fig. 4. Electronic transmission microscopy of A172 cells cultured for 7 days onto collagen gel. A172 cell on the surface of the gel, showing a smooth membrane (**A**). The arrow indicates a protrusion of the plasma membrane (**B**). CO, collagen gel; RER, endoplasmatic reticulum cisternae; MV, microvacuoles; N, nucleus.

regulated by the *RECK* product, are involved in the glioma invasiveness process, the expression of these genes was assessed, by real time PCR, as described in Experimental Procedures. T98G and A172 were cultured for two different periods of time, namely, 3 and 7 days periods, based on their growth rates (Fig. 1). These periods represent, respectively, the beginning and the full establishment of the invasive process. Since our results indicated greater differences in gene expression levels in the 7 days cultures, we focused our MMPs investigation only on this period.

The non-invasive A172 cell line displays higher levels of *RECK* (Fig. 6) and TIMP-2 expression than the invasive T98G cell line (Fig. 7B). In addition, *RECK* expression in A172 plated onto collagen is higher in samples cultured for 7 days, when compared to 3 days cultures ($P \leq 0.05$).

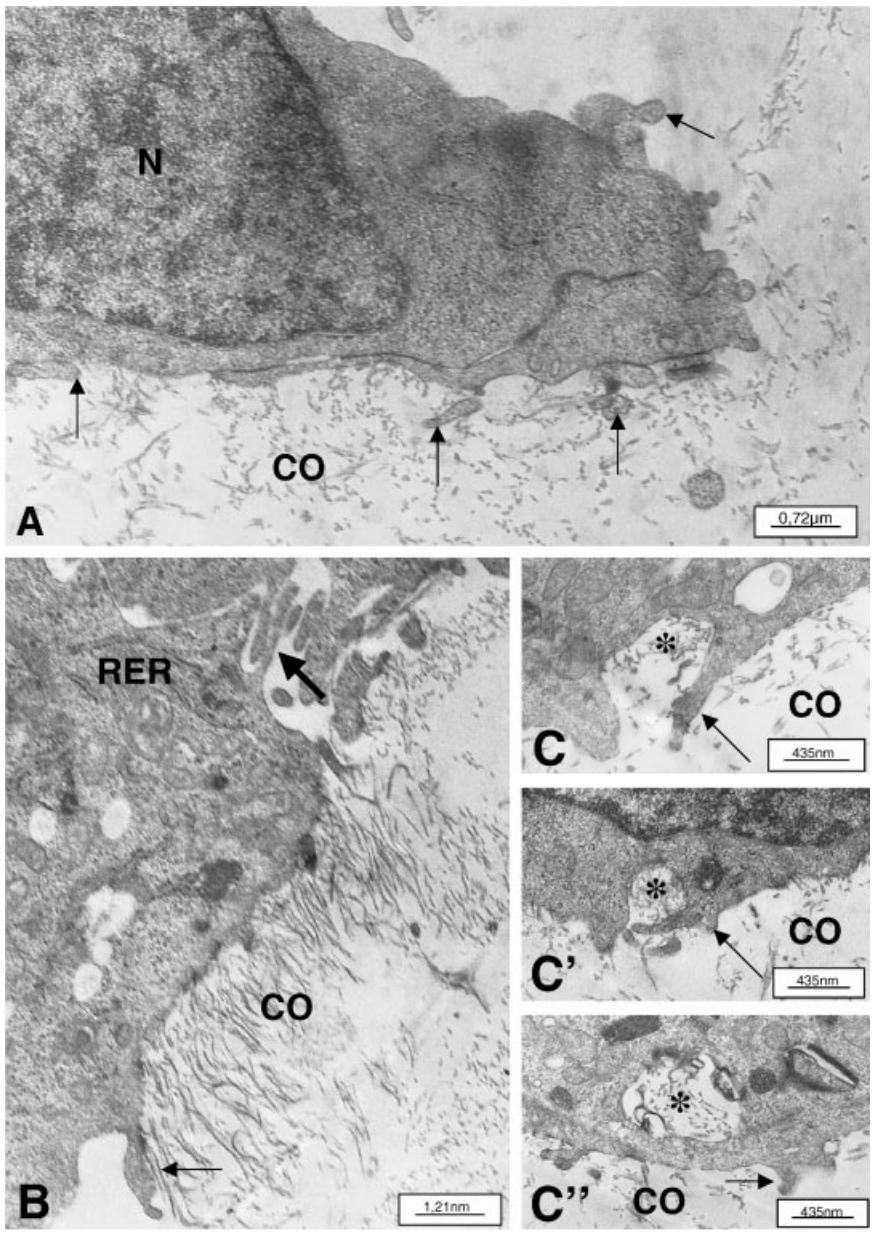

Fig. 5. Electronic transmission microscopy of T98G cells cultured for 7 days onto type 1 collagen gel. The arrows indicate several protrusions of the plasma membrane (**A**). The thicker arrow is indicating podosome-like structure (**B**). Asterisks show phagocitosis of the collagen substrate (C-C″). CO, collagen gel; RER, endoplasmatic reticulum cisternae; N, nucleus.

Opposite to what is observed for the natural MMPs inhibitors, namely, *RECK* and TIMP-2, the expression levels of MMPs-2 and -9 are higher for the invasive T98G cell line, when compared to the non-invasive A172 cell line ($P \leq 0.05$) (Fig. 7C,D). In addition, the T98G cell line displays higher expression levels of MMP-9 than A172, when cells are plated onto the collagen gel ($P \leq 0.01$). In both collagen-coated and uncoated substrates, the T98G cell line displays higher expression levels of MMP-2 than the A172 cell line ($P \leq 0.05$). In the uncoated (plastic) condition, no difference was found in the expression of MT1-MMP, which is involved in the MMP-2 activation complex, between the two cell lines (Fig. 7A). On the other hand, the collagen substrate appears as an important inducer of this membrane type MMP in A172 cells ($P \leq 0.01$).

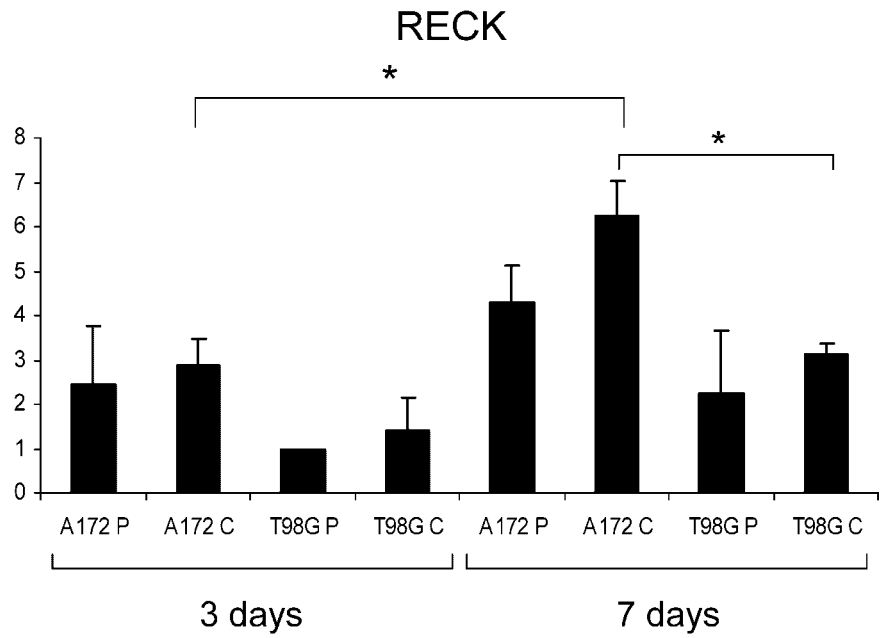

Fig. 6. Quantitative RT-PCR analysis of the *RECK* gene, showing differential expression between A172 and T98G cultured onto uncoated plastic (p) and type 1 collagen gel (c) in 3 and 7 days cultures. Note that the invasive T98G cells display lower *RECK* expression levels than the non-invasive A172 cells. The values of gene output are normalized relative to tubulin. The asterisk indicates significant difference ($P < 0.05$). Note that *RECK* levels are higher for 7 day samples.

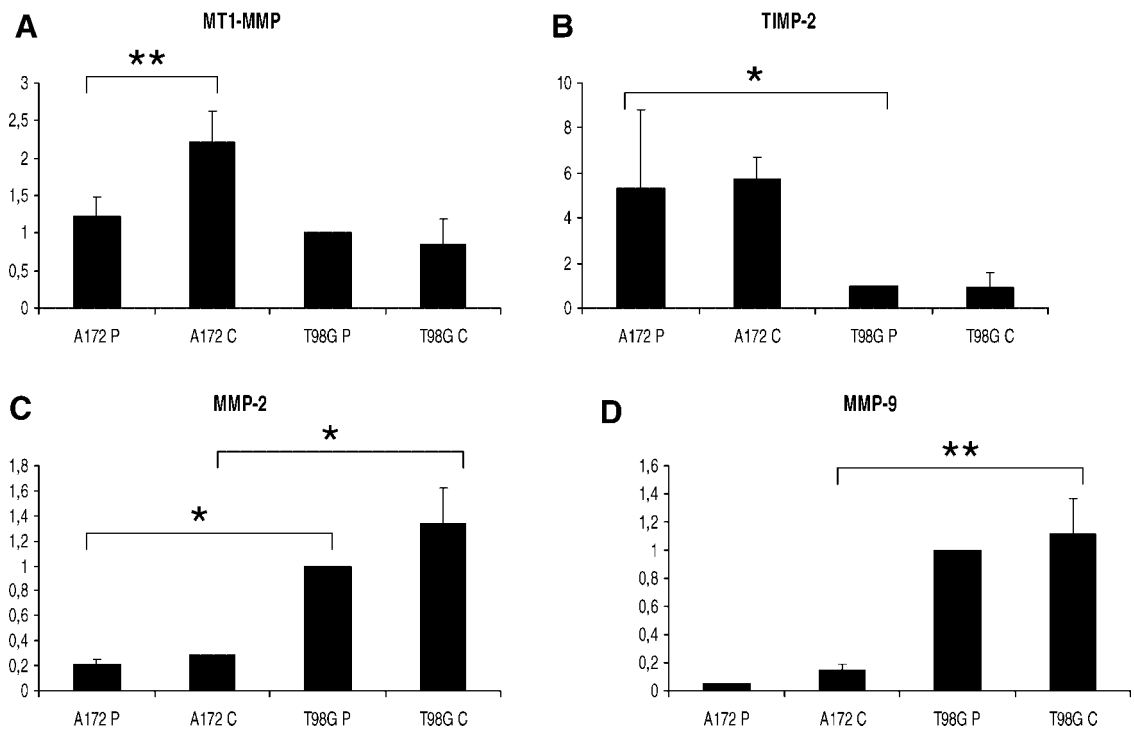

Fig. 7. Quantitative RT-PCR analysis of, MT1-MMP (**A**), TIMP-2 (**B**), MMP-2 (**C**), and MMP-9 (**D**) showing an inverse balance between MMPs and their natural inhibitor, TIMP-2. The non-invasive A172 cells, display higher expression of the inhibitor, while the invasive T98G cells, display higher levels of MMPs expression.

RECK expression is significantly induced by the collagen substrate in A172 cells (Fig. 6). On the other hand, no significant difference was found in MMPs expression when cells were cultured onto the collagen substrate (Fig. 7). It is also important to note that TIMP-2 and *RECK* expression levels are considerably higher than MMP-2, -9 and MT1-MMP.

Effect of Collagen Substrate in MMPs-2 and -9 Activity

By gelatin zymography, two different gelatinolytic activities, of, respectively, 92 and 72 kDa, were detected for both A172 and T98G cell lines, when cultured onto plastic (control) or type 1 collagen gel (Figs. 8 and 9).

This assay revealed, firstly, that MMP-2 activity levels are significantly higher for the invasive T98G cell line, when compared to the non-invasive A172 cells (Fig. 9A). On the other hand, no significant difference in MMP-9 activity was found between the A172 and the T98G cell lines (Fig. 9D). Secondly, the collagen substrate appears as an important inducer of MMP-2 activity, significantly increasing it in both cell lines (Fig. 9B,C). Also, for both cell lines, MMP-9 activity was only detectable in cultures maintained onto the collagen-coated surface, indicating that this ECM component is a strong regulator of the activity of these enzymes (Fig. 9E,F).

DISCUSSION

Malignant gliomas are the most common and aggressive primary tumors of the adult central nervous system. A recent study focusing on the mechanisms of gliomas invasion suggests that matrix metalloproteinases (MMPs) play a critical role in this process [Annabi et al., 2004]. MMPs belong to a large family of zinc-dependent natural endopeptidases, which promote tumor cells invasion by degrading extracellular matrix proteins, such as collagen, fibronectin, and proteoglicans [Coussens et al., 2002; Annabi et al., 2004].

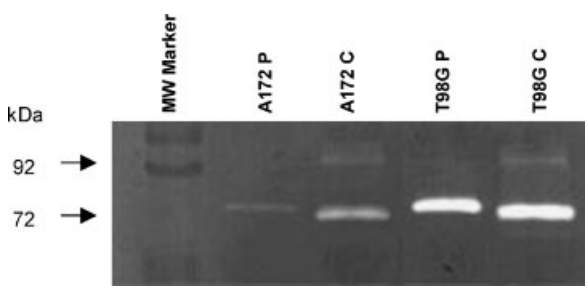

Fig. 8. MMP-2 and -9 activities assessed by gelatin zymography. MMP-2 (72 KDa) activity is much greater than MMP-9 (92 KDa), which is only detectable for cells cultured onto collagen.

Our findings suggest that *RECK*, as a potent inhibitor of MMP-2, -9 and MT1-MMP, is involved in the invasive process in the glioma model, since it is poorly expressed by the invasive T98G cells and is highly expressed in the non-invasive A172 cells. In addition, *RECK* expression inversely correlates with MMP-2 and -9 expression levels, which are higher in T98G cells, when compared to A172 cells, possibly contributing to the aggressive invasive behavior of the former.

Takahashi et al. [1998] and Oh et al. [2001] reported that *RECK* does not alter the transcriptional levels of MMPs in the HT-1080 fibrosarcoma model, but, on the other hand, *RECK* directly interacts with MMP-2 and -9 and MT1-MMP and inhibits their activities. We assayed MMP-2 and -9 activities by gelatin zymography and show that, in the absence of the collagen substrate, T98G cells display higher MMP-2 activity, when compared with A172 cells. MMP-2 and -9 activity levels are higher when T98G cells penetrate into the collagen gel, while A172 cells, which do not invade the substrate, display lower levels of these MMPs activities.

Wang et al. [2003] analyzed MMP-2 and -9 expression by immunohistochemistry, showing that no positive expression of MMP-2 and-9 was present in normal brain tissue, whereas in gliomas the positive staining for these MMPs was significantly elevated, progressively increasing with the malignancy degree, which is in agreement with our results, since T98G cells display a more aggressive behavior than A172.

Here, we show, that the collagen substrate promotes both an increase in *RECK* expression and in MMP-2 and -9 activities in both T98G and A172 glioma cells lines. This is actually the opposite of what has been described for HT-1080 fibrossarcoma cells that overexpress *RECK*, where high *RECK* expression levels are associated with lower MMPs activities [Takahashi et al., 1998; Oh et al., 2001].

Type 1 collagen is a known matrix effector for MMP-2 activation in various cell types, however, the mechanisms by which MMP-2

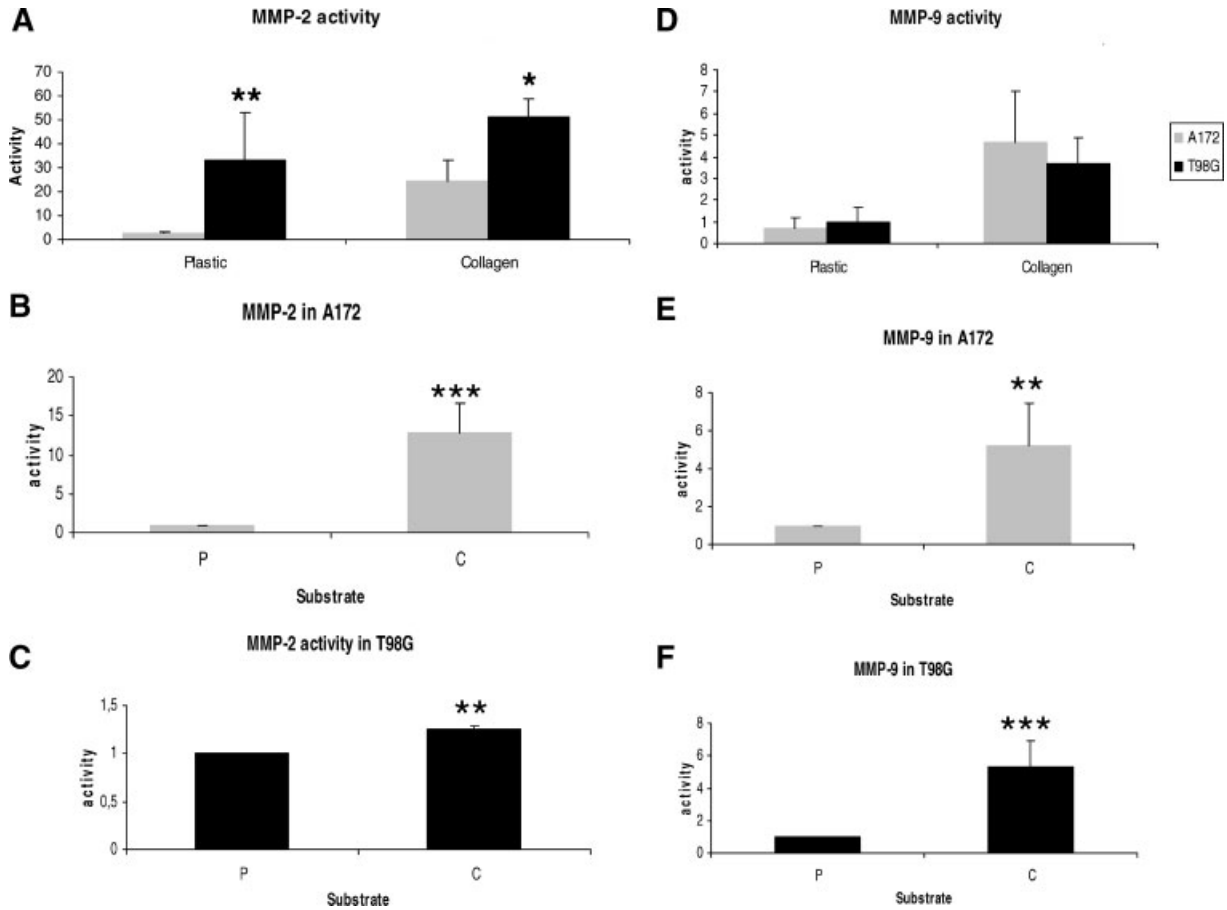

Fig. 9. Levels of MMP-2 (**A**) and MMP-9 (**D**) for both cell lines. Notice that T98G cells, which are invasive, show greater MMPs activities. Collagen substrate appears an inducer of MMP-2 and -9 activities for A172 (**B** and **E**) and also for T98G (**C** and **F**) $^{**}P < 0.01$; $^{***}P < 0.005$.

activation is induced by three-dimensional type 1 collagen remains unclear. Nevertheless, evidence has accumulated showing that expression of MT1-MMP is essential for MMP-2 activation when several cell types are embedded in type 1 collagen lattice [Guo and Piacentini, 2003].

Profound morphological changes, accompanied by MMP-2 activiation, occur when U-87 glioma cells are induced by type 1 collagen treatment [Annabi et al., 2004], in agreement with our results. These results are also in line with Giese et al. [1996], who claim that proliferation and migration are temporally and mutually exclusive events for the glioma model, as highlighted by the A172 and T98G cells growth curves (Fig. 1). The results obtained with A172 cells show that the collagen substrate caused a significant increase in MT1-MMP expression (Fig. 7A), accompanied by significant increase in MMP-2 activity (Figs. 8 and 9). Even more interestingly, we report here that, in addition to upregulation of MT1-MMP, upregulation of *RECK* expression also occurs in the collagen-coated condition.

Another important feature that we found upon culturing the invasive T98G cells onto collagen is the presence of regions similar to a recently described structure involved in ECM degradation, namely, the podosomes. These structures are typically found in cells that have to cross tissue boundaries [Linder and Aepfelbacher, 2003], such as T98G cells invading the collagen matrix. Podosomes are columnar arrays of actin filaments surrounding narrow tubular invagination of the plasma membrane roughly perpendicular to the substratum [Spinardi et al., 2004]. It has been described that podosomes contain and secrete MMPs, which is consistent with the high MMPs levels of expression and activity that we describe for T98G cells.

The TIMP family comprises four natural inhibitors of MMPs, namely: TIMPs 1–4. Therefore, we decided to evaluate TIMP-2 expression, since it takes part in the MMP-2 activation complex, which is regulated by *RECK* and an increase of MMP-2 activity was observed when cells were cultured onto collagen, for both A172 and T98G cells. In addition, differently from other TIMPs, TIMP-2 has a cell surface receptor, that mediates direct growth inhibition. Oh et al. [2004] reported another important correlation between *RECK* and TIMP-2, since TIMP-2 is able to induce *RECK* expression in hMVECs cells.

Even though *RECK* and TIMPs are both MMPs inhibitors, they may play different roles in different processes. Lack of *RECK* is embryonic lethal in mice, while lack of TIMP-1 and -2 has little effect on development, leading Oh et al. [2001] to suggest that *RECK* shares little functional redundancy with TIMPs, at least around the 10th day of development. Our results, obtained using two glioma cell lines, with different invasive properties, show that *RECK* and TIMP-2 share common features, since both display high expression levels and inhibit MMPs in this model. On the other hand, the levels of *RECK*, but not of TIMP-2, can be altered in the presence of the collagen substrate, constituting a major difference between these two MMPs inhibitors. Therefore, collagen modulation of *RECK* expression may be a useful approach to prevent glioma progression.

In conclusion, the gene expression patterns of MMPs and MMPs inhibitors seem to behave in a see-saw mode. Thus, when MMPs-2 and -9 displayed higher levels, their inhibitors, *RECK* and TIMP-2, displayed lower levels, which is consistent with the invasive and non-invasive behaviors described for, respectively, T98G and A172 glioma cell lines.

Taken together, our results could suggest that a positive feedback mechanism exists between *RECK* expression and MMP-2 and -9 activities. Thus, in the presence of collagen, MMP-2 and -9 are induced, favoring the invasive process. It is possible that higher MMPs activity causes higher *RECK* expression, counteracting their invasive effects. Higher MMPs activity may lead to *RECK* induction. This inverse balance between *RECK* and MMPs expression has been related to increased patient survival for many human tumors, such as hepatocarcinomas, breast, colorectal, prostate, and pancreatic tumors, ever since *RECK* was first described [Furumoto et al., 2001; Masui et al., 2003; Span et al., 2003; Takenaka et al., 2004; Takeuchi et al., 2004].

Elucidation of *RECK* expression and its relationship with MMPs expression and activity, may contribute to better understanding of glioma invasion and metastasis processes. Because of the complexity of intracellular signaling pathways involved in MMPs activation, whether *RECK* is their effective regulator in the glioma model is an open question.

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