Experimental
ONCOLOGY

EXPRESSION OF MEMBRANE-ANCHORED MATRIX METALLOPROTEINASE INHIBITOR *REVERSION INDUCING CYSTEINE RICH PROTEIN WITH KAZAL MOTIFS* IN MURINE CELL LINES

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Aim: It has been demonstrated that the endogenous matrix metalloproteinases (MMPs) inhibitor reversion inducing cysteine rich protein with Kazal motifs (*RECK*) is a reliable prognostic marker for detecting several types of tumors. However, the *RECK* expressions in most of the normal and neoplastic tissues were extremely low, and to measure its expression is quite complicated. The purpose of the present study is to establish an easy method to quantify murine *RECK* mRNA expression for use in future experimental studies. Subsequently, in order to verify the reliability of the established quantification technique, we examined the change in *RECK* expression and gelatinase secretion in tumor cells when stimulated by the extracellular matrix. *Methods:* Several murine tumor cells were used in the present study. The real-time polymerase chain reaction (PCR) method and measurement conditions for murine *RECK* mRNA were studied using these tumor cells. Gelatinase activities were also examined by gelatin zymography. *Results:* Murine *RECK* mRNA expression was accurately quantified using real-time PCR. Among the tumor cells used in the study, osteosarcoma cells showed significantly higher *RECK* mRNA expression than the others. The *RECK* expression in the osteosarcoma cells was down-regulated by contact with matrigel-coated culture flasks due to increased secretion of gelatinases. *Conclusion:* The real-time PCR method employed in our study is useful to quantify *RECK* expression.
Key Words: RECK, gelatinase, MMP inhibitor, real-time PCR, osteosarcoma.

Matrix metalloproteinases (MMPs) play important roles in the mechanism of tumor invasion and metastasis by selectively degrading the extracellular matrix. Among the various MMPs, gelatinases (type-IV collagenases), particularly MMP-2 and -9, are closely linked with tumor malignancy [11, 20], and the increased levels of these enzymes in tumor patients are strongly related to poor prognosis [7, 13, 14].

A novel endogenous specific MMP inhibitor, which was designated as "reversion inducing cysteine rich protein with Kazal motifs" (RECK), has been recently isolated [28]. RECK inhibits tumor invasion and tumor angiogenesis by negatively regulating MMP-2, -9, and membrane-type 1-MMP (MT1-MMP) [19, 28]. It has been suggested that this membrane-anchored glycoprotein can be considered as one of the reliable prognostic factors in several tumor patients [10, 18, 24].

In the majority of studies, the *RECK* mRNA expression levels were quantified by Northern blotting [10, 18, 28]. However, the *RECK* mRNA expression levels were reported to be considerably low in most of the normal human tissues and tumor cells [28]. Therefore, to detect the low expression levels of the *RECK* mRNA by Northern blotting, a large volume of specimens was required to purify sufficient amounts of the mRNA. In view of this, the quantitative real-time polymerase chain reaction (PCR) analysis is advantageous because it allows detection of the mRNA even in small amounts of specimens, and it is suitable for treating a large number of samples. Real time PCR had been used to quantify human and canine *RECK* mRNA expression, and the amplification condition was established [24, 26, 27]. However, this technique has not been established for murine *RECK*.

The purpose of the present study was to investigate the *RECK* expression levels in several murine tumor cells by employing the quantitative PCR. Subsequently, in order to confirm the reliability of the established quantification technique and correlation of gelatinase activity, the change in *RECK* expression and gelatinase secretion was studied in tumor cells attached to the extracellular matrix.

MATERIALS AND METHODS

Murine cell lines and cell culture. The following mouse tumor cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 1,000 U/ml of penicillin, 0.1 mg/ml streptomycin, and 10% fetal bovine serum (FBS) at 37 °C and 5% CO_2 condition: B16 (melanoma) [12], B16F1 (pulmonary metastatic melanoma derived from B16) [23], P815 (mast cell tumor) [8], YAC-1 (lymphoma) [15], KLN205 (squamous cell carcinoma) [22], S-180 (sarcoma) [31], Dunn (osteosarcoma) [21], LM8 (pulmonary metastatic melanoma derived from Dunn) [1].

RNA extraction and reverse transcription. Each tumor cell line was washed twice and incubated for 24 h in a culture medium lacking FBS. Then, total RNA was extracted by the guanidine isothiocyanate method [5]. The total RNA (2 μg) was denatured at 70 °C for 10 min, immediately cooled, and added to a solution containing 200 units of M-MLV reverse transcriptase (Invitrogen, USA), 50 nmol dithiothreitol, 10 pmol poly (dT) primer, and 20 nmol dNTPs in a total volume of 20 μl. After the poly (dT) primer was annealed at 20 °C for 10 min, cDNA synthesis was performed at 37 °C for 1 h.

Quantitative PCR analysis of RECK gene expression in mouse tumor cell lines. All PCR reactions were performed using a detection kit (Lightcycler-FirstStart DNA Master SYBR Green kit, Roche Molecular Biochemicals, Mannheim, Germany). The primer pairs used for amplifying RECK mRNA were as follows: sense, 5′-GCA TGC AAG CAG GCA TCT TC-3′ and antisense, 5′-CTG TGG ACT GAT AGA GGC AC-3′. To ensure correctness of mRNA extraction and reverse-transcription and standardize the samples, all the samples were subjected to PCR amplification using oligonucleotide primers specific for the constitutively expressed gene glyceraldehydes-3-phoshate-dehydrogenase (GAPDH). The primer pairs for amplifying GAPDH mRNA were as follows: sense, 5′-GAA GGT CGG TGT GAA CGG ATT-3′ and antisense, 5′-GAA GAC ACC AGT AGA CTC CAC GAC ATA-3′.

The PCR reaction mixture containing the following reaction components was prepared to the indicated end concentration: 13.4 µl water, 2.4 µl $MgCl_2$ (4 mM), 0.1 µl forward primer (100 µM), 0.1 µl reverse primer (100 µM), and 2.0 µl LightCycler Fast Start DNA Master SYBR Green I (Roche Molecular Biochemicals). As a PCR template, 18 µl of master-mix and 2 µl of cDNA as a PCR template were added to glass capillaries.

The cycling conditions were as follows: initial denaturation at 95 °C for 30 s, followed by 35 cycles (*RECK*) or 30 cycles (*GAPDH*) at 95 °C for touchdown (0 s), 60 °C for 5 s, and 72 °C for 10 s. In order to improve the SYBR Green I quantification, the temperature of the fluorescence measurement point was set at 84 °C (*RECK*) and 83 °C (*GAPDH*). The expression level of each mRNA in the tissue samples was determined relative to the standard curve by using the LightCycler computer software (Roche Molecular Biochemicals). The identity of each PCR product was confirmed by electrophoresis.

Stimulation by extracellular matrix. Matrigel-coated plates were used to stimulate Dunn and LM8 cells by direct contact with the extracellular matrix. Matrigel (BD Biosciences, USA) is composed of the extracellular matrix that is secreted by Engelbreth-Holm-Swarm mouse sarcoma cells. Matrigel was diluted to 0.1 mg/ml with phosphate-buffered saline (PBS) on ice. Subsequently, 1 ml of the solution was poured into each well of the 6-well cell culture plates. These solutions were dried by leaving plates overnight on a clean bench under an air flow. Cell culture medium containing 10% FBS (2 ml) was poured 2 h before seeding it with the cell suspension solution (2 ml). The concentration of cells in the culture medium was adjusted to 1.0×10^6 cells/ml. After 24 h of incubation, cells were washed twice with serum free medium. The collection of RNA from each cell line and culture medium was performed after 24 h.

Gelatin zymography. Gelatin zymography was employed to investigate the correlation between murine RECK mRNA expression and gelatinize activity. By using the previously reported method, gelatin zymography was performed [2, 29]. Briefly, 20 µl of the supernatant of each conditioned medium was mixed with an equal volume of 2 x SDS-PAGE sample buffer without boiling. The samples were fractionated by electrophoresis on a 10% polyacrylamide gel containing gelatin (0.5 mg/mL). In order to remove the SDS, the gels were soaked in 2.5% Triton X-100 containing 10 mM Tris (pH 8.0) for 30 min at room temperature and incubated in a digestion buffer (50 mM Tris [pH 8.0], 0.5 mM $CaCl_2$, and 1 µM $ZnCl_2$) at 37 °C for 30 h to enable the proteinase digestion of the substrate. The gels were stained with 0.25% Coomassie brilliant blue R-250 and destained with 10% isopropanol and 5% acetic acid. The gelatinolytic activities were observed due to the appearance of clear bands of digested gelatin against a dark blue background of stained gelatin.

Statistical analysis. For the comparison of the murine RECK expression levels in tumor cells, analysis of variance followed by Scheffe's multiple comparison as the post-hoc test was performed. For the comparison of control and stimulated cells, the Student's *t*-test was used. Differences were considered significant when $p < 0.05$. The statistical analyses were performed using the Stat View 5.0 software (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

***RECK* gene expression in murine tumor cells.** Quantitative real time PCR could accurately measure the amount of *RECK* mRNA, and the amplified cDNA completely corresponded with a part of the reported murine *RECK* gene (Fig. 1). Northern blotting was used in most of the previous studies to quantify the expression level of the *RECK* mRNA [10, 18, 28]. The findings of these studies were that the *RECK* mRNA expression level in normal human tissues and tumor cells was considerably low. Therefore, to detect the low *RECK* mRNA expression by Northern blotting, it was necessary to purify the poly A (+) mRNA from the total RNA and obtain an adequate number of samples. The quantitative PCR used in this study indicated that this was an easy, useful, and sensitive technique to quantify the mRNA levels of specific genes. Moreover, the process did not involve a treatment step for purifying the mRNA from total RNA.

Slope = –3.048
Intercept = 41.20
Error = 0.181
r = –1.00

$y = –3.4075x + 41.19^3$
$R^2 = 1$
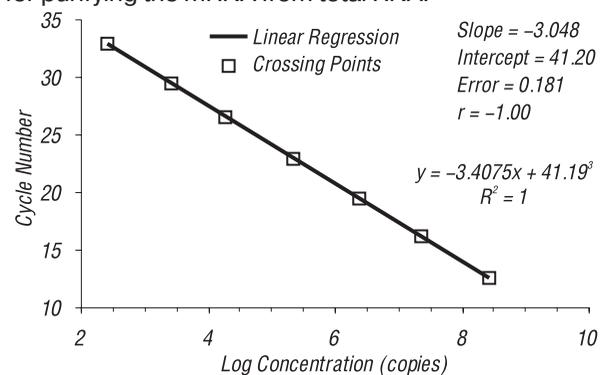
Fig. 1. Standard curve for quantification of murine reversion inducing cysteine rich protein with Kazal motifs (*RECK*) mRNA. All measurements of murine *RECK* was performed when the error value was less than 0.2

The murine tumor cells showed variable levels of murine *RECK* gene expression (Fig. 2). Although most cell lines showed extremely low *RECK* mRNA expression, two osteosarcoma cell lines showed a clear expression and

the S-180 cell — line comparatively higher *RECK* mRNA expression. The previous studies reported that there was little *RECK* mRNA expression in the murine and human tumor cells [28]. However, in canine osteosarcoma cell line, the high *RECK* mRNA expression was confirmed [26]. In the present study, murine osteosarcoma was found to exhibit significantly higher levels of *RECK* mRNA compared to other tumor cell lines. The highly metastatic LM8 cells derived from the Dunn osteosarcoma cell line showed significantly lower expression than parental cell line. As it was reported [28], the more malignant tumor cells exhibited lower *RECK* mRNA expression compared to parental cell lines. In particular, B16F1 and LM8 cells, more metastatic than the cell lines that they are derived from (B16 and Dunn, respectively) [1, 23], are showed to possess lower *RECK* mRNA expression.
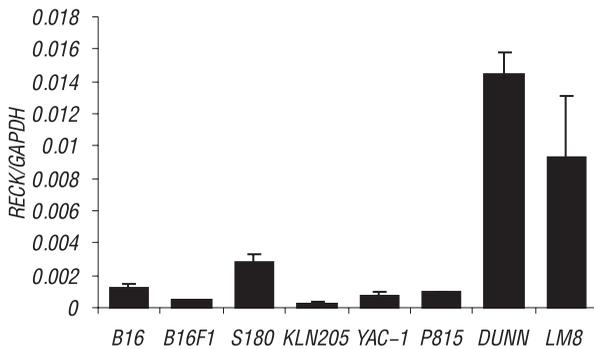
Fig. 2. Murine *RECK* gene expression in various types of tumor cells. GAPDH: glyceraldehyde-3-phoshate-dehydrogenase

Regulation of RECK mRNA expression under extracellular matrix stimulation. Osteosarcoma cells that were cultured on matrigel-coated plates were attached to the bottom of the plates in a manner similar to that observed on standard culture plates. However, the cells exhibited different morphology depending on the extracellular matrix present in the culture plates. The tumor cells cultured on matrigel-coated plates were fibroblastic, although the cells appeared to be osteoblastic when cultured on standard plates. The *RECK* mRNA expression in the cells grown on matrigel was significantly lower (by 2/3 nad ½ in Dunn and LM8 cells respectively) than that in the cells grown in standard conditions (Fig. 3).
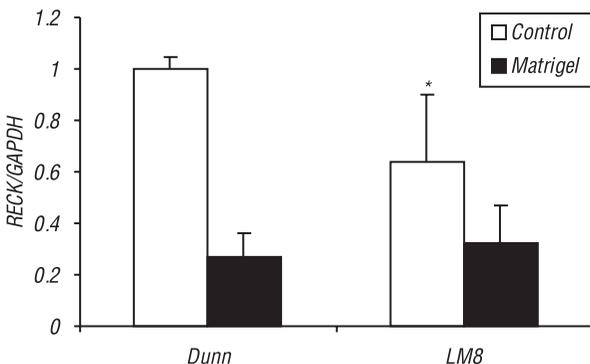
Fig. 3. The *RECK* gene expression in the osteosarcoma cell lines upon contact with matrigel. The *RECK* expression in Dunn cells grown in the matrigel-coated flask was significantly lower than that in control flasks (*p < 0.05). LM8 cells also showed changes identical to that shown by Dunn

There was one report stating that type-1 downregulation of the *RECK* expression in human glioma cells grown on collagen-gel coated plate [6]. However, our data (unpublished) indicated that type-1 collagen did not cause *RECK* downregulation in the murine osteosarcoma. In the present report, *RECK* expression in Dunn and LM8 cells was downregulated due to direct contact with matrigel — an extracellular matrix secreted by the Engelbreth-Holm-Swarm mouse sarcoma [16]. The requirement for direct contact of the cells with an extracellular matrix to induce *RECK* downregulation might be due to the fact that RECK is a membrane-anchored protein.

Gelatin zymography. In the present study, gelatin zymography was performed to validate the correlation between *RECK* downregulation and gelatinase activities and secretion. The MMPs are secreted from several types of cells as inactivated proenzymes that are subsequently activated by other MMPs or serine proteinases [9]. Gelatin zymography is a method that enables visualizing the amount of gelatinases as clear bands. This technique is distinct from Western blotting and enzyme-linked immunosorbent assay (ELISA) [3] because it can measure not only activated gelatinases but also simultaneously measure inactivated pro-MMPs [4, 30]. RECK is a membrane-anchored protein that negatively regulates secretion and activities of gelatinases. Thus, the data obtained by measuring gelatinase activity can be used for evaluation of RECK expression.

Initially, zymographic analysis was carried out on the same medium on which the Dunn osteosarcoma cells were cultured without any stimulation. No visible band indicating the secretion of pro-MMP-2 and pro-MMP-9 was observed (Fig. 4). LM8 cells secreted larger amounts of pro-MMP-2 in their conditioned medium, and this result was consistent to that of a previous report [1]. When these tumor cells are in contact with matrigel, they exhibited increased gelatinases secretion. The contact of the cells with matrigel induced secretion of MMP-9 in Dunn cells and secretion of both the gelatinases MMP-2 and 9 in LM8 cells. These results might indicate a close correlation between the increased gelatinase secretion and downregulation of murine *RECK* mRNA.
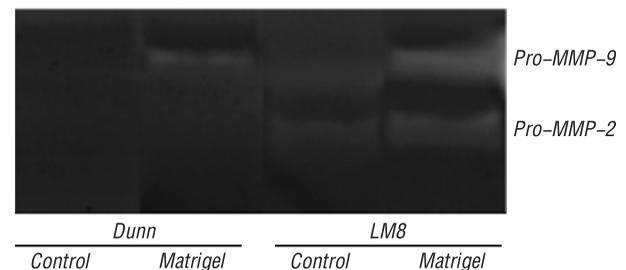
Fig. 4. Gelatinases activity observed in the two types of osteosarcoma cells stimulated by matrigel. Gelatinase activation was induced due to direct contact with matrigel. MMP: Matrix metalloproteinase

It is known that the level of gelatinase activity is altered in tumor tissues [1, 10, 18, 25]. Several reports have shown that gelatinases can be upregulated or activated in osteosarcoma cells [17, 20]. In the present study, we have shown that direct contact with matrigel induced MMP secretion and *RECK* downregulation.

REFERENCES

1. **Asai T, Ueda T, Itoh K, Yoshioka K, Aoki Y, Mori S, Yoshikawa H.** Establishment and characterization of a murine osteosarcoma cell line (LM8) with high metastatic potential to the lung. Int J Cancer 1998; **76**: 418−22.

2. **Becerril C, Pardo A, Montano M, Ramos C, Ramirez R, Selman M.** Acidic fibroblast growth factor induces an antifibrogenic phenotype in human lung fibroblasts. Am J Respir Cell Mol Biol 1999; **20**: 1020−7.

3. **Chen F, Eriksson P, Hansson GK, Herzfeld I, Klein M, Hansson LO, Valen G.** Expression of matrix metalloproteinase 9 and its regulators in the unstable coronary atherosclerotic plaque. Int J Mol Med 2005; **15**: 57−65.

4. **Chen Y, Hozawa S, Sawamura S, Sato S, Fukuyama N, Tsuji C, Mine T, Okada Y, Tanino R, Ogushi Y. Nakazawa H.** Deficiency of inducible nitric oxide synthase exacerbates hepatic fibrosis in mice fed high-fat diet. Biochem Biophys Res Commun 2004; **326**: 45−51.

5. **Chomczynski P, Sacchi N.** Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; **162**: 156−9.

6. **Correa TC, Brohem CA, Winnischofer SM, da Silva Cardeal LB, Sasahara RM, Taboga SR, Sogayar MC, Maria-Engler SS.** Downregulation of the RECK-tumor and metastasis suppressor gene in glioma invasiveness. J Cell Biochem 2006; **99**: 156−67.

7. **Dalberg K, Eriksson E, Enberg U.** Gelatinase A, membrane type 1 matrix metalloproteinase, and extracellular matrix metalloproteinase inducer mRNA expression: correlation with invasive growth of breast cancer. World J Surg 2000; **24**: 334−40.

8. **Demehri S, Corbin A, Loriaux M, Druker BJ, Deininger MW.** Establishment of a murine model of aggressive systemic mastocytosis/mast cell leukemia. Exp Hematol 2006; **34**: 284−8.

9. **Foda HD, Zucker S.** Matrix metalloproteinases in cancer invasion, metastasis and angiogenesis. Drug Discov Today 2001; **9**: 478−82.

10. **Furumoto K, Arii S, Mori A, Furuyama H, Gorrin Rivas MJ, Nakao T, Isobe N, Murata T, Takahashi C, Noda M, Imamura M.** RECK gene expression in hepatocellular carcinoma: correlation with invasion-related clinicopathological factors and its clinical significance. Reverse-inducing-cysteine-rich protein with Kazal motifs. Hepatology 2001; **33**: 189−95.

11. **John A, Tuszynski G.** The role of matrix metalloproteinases in tumor angiogenesis and tumor metastasis. Pathol Oncol Res 2001; **7**: 14−23.

12. **Kaliski A, Maggiorella L, Cengel KA, Mathe D, Rouffiac V, Opolon P, Lassau N, Bourhis J, Deutsch E.** Angiogenesis and tumor growth inhibition by a matrix metalloproteinase inhibitor targeting radiation-induced invasion. Mol Cancer Ther 2005; **4**: 1717−28.

13. **Kawamata H, Uchida D, Hamano H.** Active-MMP2 in cancer cell nests of oral cancer patients: correlation with lymph node metastasis. Int J Oncol 1998; **13**: 699−704.

14. **Kawano N, Osawa H, Takaaki I.** Expression of gelatinase A, tissue inhibitor of metalloproteinases-2, matrilysin, and trypsin(ogen) in lung neoplasms. Hum Pathol 1997; **28**: 613−22.

15. **Kim GY, Lee JY, Lee JO, Ryu CH, Choi BT, Jeong YK, Lee KW, Jeong SC, Choi YH.** Partial characterization and immunostimulatory effect of a novel polysaccharide-protein complex extracted from *Phellinus linteus*. Biosci Biotechnol Biochem 2006; **70**: 1218−26.

16. **Kleinman HK, McGarvey ML, Liotta LA, Robey PG, Tryggvason K, Martin GR.** Isolation and characterization of type IV procollagen, laminin, and heparan sulfate proteoglycan from the EHS sarcoma. Biochemistry 1982; **21**: 6188−93.

17. **Kurogi T, Nabeshima K, Kataoka H, Okada Y, Koono M.** Stimulation of gelatinase B and tissue inhibitors of metalloproteinase (TIMP) production in co-culture of human osteosarcoma cells and human fibroblasts: gelatinase B production was stimulated via up-regulation of fibroblast growth factor (FGF) receptor. Int J Cancer 1996; **66**: 82−90.

18. **Masui T, Doi R, Koshiba T, Fujimoto K, Tsuji S, Nakajima S, Koizumi M, Toyoda E, Tulachan S, Ito D, Kami K, Mori T, Wada M, Noda M, Imamura M.** RECK expression in pancreatic cancer: its correlation with lower invasiveness and better prognosis. Clin Can Res 2003; **9**: 1779−84.

19. **Oh J, Takahashi R, Kondo S, Mizoguchi A, Adachi E, Sasahara RM, Nishimura S, Imamura Y, Kitayama H, Alexander DB, Ide C, Horan TP, Arakawa T, Yoshida H, Nishikawa S, Itoh Y, Seiki M, Itohara S, Takahashi C, Noda M.** The membrane-anchored MMP inhibitor RECK is a key regulator of extracellular matrix integrity and angiogenesis. Cell 2001; **107**: 789−800.

20. **Okada Y, Tsuchiya H, Shimizu H, Tomita K, Nakanishi I, Sato H, Seiki M, Yamashita K, Hayakawa T.** Induction and stimulation of 92-kDa gelatinase/type IV collagenase production in osteosarcoma and fibrosarcoma cell lines by tumor necrosis factor alpha. Biochem Biophys Res Commun 1990; **171**: 610−7.

21. **Okamoto T, Yamada N, Tsujimura T, Sugihara A, Nishizawa Y, Ueda H, Kashiwamura S, Tsutsui H, Futani H, Maruo S, Okamura H, Terada N.** Inhibition by interleukin-18 of the growth of Dunn osteosarcoma cells. J Interferon Cytokine Res 2004; **24**: 161−7.

22. **O-Sullivan I, Ng LK, Martinez DM, Kim TS, Chopra A, Cohen EP.** Immunity to squamous carcinoma in mice immunized with dendritic cells transfected with genomic DNA from squamous carcinoma cells. Cancer Gene Ther 2005; **12**: 825−34.

23. **Sloane BF, Honn KV, Sadler JG, Turner WA, Kimpson JJ, Taylor JD.** Cathepsin B activity in B16 melanoma cells: a possible marker for metastatic potential. Cancer Res 1982; **42**: 980−6.

24. **Span PN, Sweep CG, Manders P, Beex LV, Leppert D, Lindberg RL.** Matrix metalloproteinase inhibitor reversion-inducing cysteine-rich protein with Kazal motifs: a prognostic marker for good clinical outcome in human breast carcinoma. Cancer 2003; **97**: 2710−5.

25. **Stetler-Stevenson WG.** The role of matrix metalloproteinases in tumor invasion, metastasis, and angiogenesis. Surg Oncol Clin N Am 2001; **10**: 383−92.

26. **Takagi S, Kato Y, Asano K, Ohsaki T, Bosnakovski D, Hoshino Y, Okumura M, Kadosawa T, Fujinaga T.** Matrix metalloproteinase inhibitor RECK expression in canine tumors. J Vet Med Sci 2005; **67**: 761−7.

27. **Takagi S, Kitamura T, Hosaka Y, Ohsaki T, Bosnakovski D, Kadosawa T, Okumura M, Fujinaga T.** Molecular cloning of canine membrane-anchored inhibitor of matrix metalloproteinase, RECK. J Vet Med Sci 2005; **67**: 385−91.

28. **Takahashi C, Sheng Z, Horan TP, Kitayama H, Maki M, Hitomi K, Kitaura Y, Takai S, Sasahara RM, Horimoto A, Ikawa Y, Ratzkin BJ, Arakawa T, Noda M.** Regulation of matrix metalloproteinase-9 and inhibition of tumor invasion by the membrane-anchored glycoprotein RECK. Proc Natl Acad Sci USA 1998; **95**: 13221−6.

29. **Tsuruda T, Boerrigter G, Huntley BK, Noser JA., Cataliotti A, Costello-Boerrigter LC, Chen HH, Burnett Jr JC.** Brain natriuretic peptide is produced in cardiac fibroblasts and induces matrix metalloproteinases. Circ Res 2002; **91**: 1127−34.

30. **Winnica DE, Novella ML, Dematteis A, Coronel CE.** Trypsin/acrosin inhibitor activity of rat and guinea pig caltrin proteins. Structural and functional studies. Biol Reprod 2000; **63**: 42−8.

31. **Yuan H, Song J, Li X, Li N, Dai J.** Immunomodulation and antitumor activity of kappa-carrageenan oligosaccharides. Cancer Lett 2006; **243**: 228−34.

ЭКСПРЕССИЯ ИНГИБИТОРА МЕМБРАНОСВЯЗАННЫХ МАТРИКСНЫХ МЕТАЛЛОПРОТЕИНАЗ RECK В ЛИНИЯХ ОПУХОЛЕВЫХ КЛЕТОК МЫШИ

Показано, что эндогенный ингибитор матриксных протеиназ (ММП) *RECK* **может служить надежным прогностическим маркером для некоторых типов опухолей, однако его экспрессия в большинстве нормальных и неопластических тканей крайне низкая, поэтому возникают сложности, связанные с детекцией таковой.** *Цель* **работы — разработка количественного метода определения экспрессии мРНК для использования в экспериментальных исследованиях. Для дальнейшего подтверждения надежности разработанного метода исследованы изменения экспрессии** *RECK* **и секреции желатиназ в опухолевых клетках при стимуляции внеклеточным матриксом.** *Методы:* **в работе использовали несколько линий опухолевых клеток мыши, в которых экспрессию мРНК** *RECK* **анализировали методом ПЦР в режиме реального времени, активность желатиназ — методом зимографии.** *Результаты:* **экспрессию мРНК** *RECK* **количественно оценили методом ПЦР в режиме реального времени, причем среди исследованных клеточных линий наиболее высокий уровень экспрессии** *RECK* **выявили в клетках остеосаркомы. Экспрессия** *RECK* **в клетках остеосаркомы подавлялась при контакте с культуральным пластиком, обработанным матригелем, вследствие повышения секреции желатиназ.** *Выводы:* **для количественной оценки экспрессии мРНК** *RECK* **может быть использован метод ПЦР в режиме реального времени.**
Ключевые слова: **RECK, желатиназа, ингибитор ММП, метод ПЦР в режиме реального времени, остеосаркома.**