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Review

The *RECK* Gene and Biological Malignancy–Its Significance in Angiogenesis and Inhibition of Matrix Metalloproteinases

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Abstract. *The RECK (reversion-inducing cysteine-rich protein with Kazal motifs) gene is a relatively newly discovered gene with important implications in cancer biology. RECK is normally expressed in all cells of the body and has an important role in the balance between destructive and constructive features of the extracellular matrix (ECM). The RECK protein is a membrane-bound glycoprotein that inhibits matrix metalloproteinases with the function of breaking-down the ECM. There is a significant correlation between RECK gene expression and the formation of new vessels, presumably via the mediation of vascular endothelial growth factor (VEGF), which is an important and powerful inducer of angiogenesis. Research has shown that down-regulation of RECK is caused by the rat sarcoma oncogene (RAS), which is also a common cause of tumor development in the early stages. For a tumor to progress and gain characteristics that classifies it as malignant, the degradation of the ECM and mobilization of new blood vessels are essential functions. If the tumor is inhibited with respect to these functions, it will cease to grow. RECK is, therefore, a potential tumor inhibitor but also a prognostic marker available at early clinical stages.*

The behavior of normal cells is regulated by a variety of signals. Broadly speaking, when an external signal contacts a cell, the transcription of genes that encode proteins necessary for the intracellular mediation of the specific message is elicited (1). The activation of such early genes may eventually result in a biological response, such as proliferation, differentiation, locomotion or even cell death. Every normal cell is under tight control, which basically lays down the principles for its behavior. However, when a cell becomes transformed, it ceases to respond to these regulatory signals. This is mainly because genes responsible for growth inhibition or induction of apoptosis, for example, do not operate in accordance with control procedures. Early genes can either be up-regulated or down-regulated and result in dysfunction, leading to overexpression or silencing of other regulatory genes. Gene silencing is sometimes the result of altered DNA methylation, *i.e.* when a methyl group is connected to a CG base motif thereby creating a non-readable frame (2). Up-regulation can be the result of an increased signal from another gene or a neighboring cell affecting the activity of a particular gene promoter. When these errors occur, transformed cells can grow and divide in an uncontrolled manner, invading normal tissue and leading to metastasis throughout the body.

Recently the gene *RECK* (reversion-inducing cysteine-rich protein with Kazal motifs), named after its structural features and activity (3), has been highlighted because of its correlation with metastasis and invasiveness (4-7). The RECK protein is a membrane-anchored glycoprotein which is present in all mammalian cells. The protein has its main function in tissue remodeling and thereby controls the activity of remodeling enzymes such as matrix metallo-proteinases (MMPs), especially MMP9 (3). This quality is important in wound healing and other physiological features. The level of *RECK* expression was also correlated with the biological phenotype of different cancer types (8). *RECK* was found to be down-regulated in tumors which were invasive and metastatic. Several tumor types have been tested for their RECK expression levels and there was clear correlation between RECK expression and the biological malignancy of liver, pancreatic, breast, colonic and lung cancer, as well as of melanoma and fibrosarcoma. *RECK* acts as a suppressor gene which inhibits angiogenesis, invasion

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and metastasis. However, multivariate analysis showed that the methylation status of the *RECK* gene was an important independent prognostic factor affecting overall survival of patients with cancer (p=0.037). This suggests that *RECK* is a promising biomarker in the early detection of cancer (9).

Gene Structure

Kazal-like serine protease inhibitors are defined by a conserved sequence motif. A typical Kazal domain contains six cysteine residues leading to three disulphide bonds with a 1-5/2-4/3-6 pattern. Most Kazal domains described so far belong to this class (10). The *RECK* gene was initially isolated as a suppressor gene encoding a novel membrane-anchored glycoprotein and was later found to suppress tumor invasion and metastasis by regulating MMP9. Its expression is ubiquitous in normal tissues, but undetectable in many tumor cell lines and in fibroblastic lines transformed by various oncogenes. The human *RECK* gene is located on chromosome region 9p13->p12 (3). The assignment to this chromosomal region is particularly illuminating since several potential tumor-suppressor genes are located on chromosome 9. Most importantly, among these is $p16^{INK4A}$, which plays a particular role in melanoma (11). The human *RECK* gene spans across a 87 kb region and contains 21 exons and 20 introns, with 13 single nucleotide polymorphisms (SNPs). Four SNPs were identified in the coding region of the gene (exons 1, 9, 13 and 15), and the remaining nine in introns 5, 8, 10, 12, 15 and 17. Within the coding sequence lies four of these SNPs, which increase the opportunity of disease implication. Polymorphisms lead to morbid function in the RECK protein structure (11). There is a highly homologous murine counterpart located on mouse chromosome 4 that consists of 19 exons and 18 introns. However, the *RECK* gene appears to be conserved throughout development. Recently, a mature cDNA clone from *Xenopus laevis* of the RECK gene was generated. RECK expression appeared to be low during gastrulation but increased during neurolation and into organogenesis. Furthermore, *RECK* was localized to the anterior and dorsal sides of the developing embryo (12).

Protein Structure

The RECK protein consists of 971 amino acids and both its human and mouse cDNA share 93.0% identity with each other (3). The *RECK* gene is also conserved in chimpanzee, Rhesus monkey, cow, rat, chicken, fruit fly, and mosquito (13).

The protein (Figure 1) is cysteine-rich (9%) and includes hydrophobic regions in both ends, the NH_2-terminal and COOH-terminal. The NH_2-terminal acts as a signal peptide, while the COOH-terminal operates as a signal for glycosylphosphatidylinositol (GPI) anchoring. At the center of the RECK protein there are three serine protease inhibitor-like (SPI) domains. The initial parts of the middle domain correspond to the Kazal motif, while the second and third do not (3). These SPIs are believed to inhibit the protease activity, either by physical 'trapping' or by 'reversible tight binding'. SPIs most probably play an important role in the inhibition of MMPs (11). There are also two regions with epidermal growth factor (EGF)-like repeats in the middle domain space of the protein. One site is located in the first third of the protein sequence; this is marked by five cysteine repeats which have a particular functional value, as well containing several glycosylation sites at asparagine residues. These sites are essential for obtaining proper interaction with MMP9 and MMP2, the core function of the RECK protein (11).

The RECK COOH-terminal hydrophobic region and glycophophatidylinositol (GPI) interaction are both anchored to the cell membrane. The fact that RECK is membrane-anchored is probably essential for intracellular signal transduction.

The primary function of RECK is to serve as an important mediator of tissue remodeling. RECK inhibits MMP2 and MMP9 and metallothionein-1 (MT1)–MMP post-transcriptionally. MMP2 and MMP9 break-down ECM, both under normal conditions and during pathological conditions. Normally RECK is expressed in all human and mammalian cells, including mesenchymal tissues and in vascular smooth muscle cells (14).

In a study by Takahashi *et al.*, it was confirmed that activated *RAS* oncogenes suppress the *RECK* promoter (3). Down-regulation of *RECK* mRNA was also induced by a variety of other oncogenes, such as viral feline osteosarcoma (*v-FOS*), myelocytomatosis (*c-MYC*), Rous sarcoma (*v-SRC*), McDonough feline sarcoma (*v-FMS*), feline sarcoma (v-FES) and viral murine osteosarcoma (*v-MOS*) oncogenes (3). Dramatic morphological alternations developed as a result of mutated *RAS* genes, which occurs often in many kinds of tumors (15).

KO mice with a disrupted *RECK* gene were generated in order to understand its physiological significance (14). Embryos of $RECK^{-/-}$ mice were much smaller in body size, had a reduction in structural integrity and frequent abdominal hemorrhage. A histological analysis also revealed severe disorganization of mesenchymal tissues and terminated organogenesis in the mutant embryonic mice. Moreover, vascular endothelial cells did not form tight and thin tubular structures as seen in the wild-type embryos, but rather displayed primitive vascular plexuses, which suggests deficient blood maturation rather than vasculogenesis. Recently, much interest has centered on the role of nuclear receptors in the regulation of *RECK* expression. The farnesoid receptor (FXR) is a ligand-activated nuclear receptor which controls the expression of genes involved in glucose metabolism, as well as in bile acid homeostasis and lipoprotein metabolism. FXR acts directly on the *RECK* gene by binding to an FXR response element located on the first

intron of the RECK gene. As a result, *RECK* expression is enhanced (16). By contrast, it was found that *RECK* transcript levels were decreased by binding of estrogen to the ER and unaffected by binding of progesterone to the PR (17).

The Physiological Role of RECK in Animal Experiments

To be able to understand the main functions of RECK, there have been several studies carried out *in vivo* (14, 18). Mice with a defective RECK gene have been studied. Heterozygous mice, with a RECK mutation ($RECK^{-/+}$) were healthy and fertile, but they did not give birth to any homozygous offspring ($RECK^{-/-}$). A ratio of about 1:2 of wild-type to heterozygous offspring indicated a recessive lethal phenotype. Thus, embryos were examined from heterozygous intercrosses at different developmental stages. No obvious phenotype was seen in $RECK^{-/-}$ embryos at an early stage. However, two-thirds of the embryos died due to the lack of heartbeat. Later in embryonic development, none of the $RECK^{-/-}$ embryos survived. These embryos differed from embryos with a functional RECK since they had smaller body size, a prominent reduction in structural integrity and recurring abdominal hemorrhage. This was revealed by histological examination of the mutant embryos, where the results displayed a severe disarray of mesenchymal tissues and disrupted organogenesis. However, most importantly, the vascular endothelial cells did not form tight and tubular structures as they ought to. The vascular network that developed in the yolk sac resembled primitive vascular plexuses, which suggests defects in blood vessel maturation, rather than vasculogenesis (14).

A study on Syrian golden hamsters was performed, where the hamsters were divided into two groups (18): group 1 remained untreated and group 2 was infected with virus to develop cholangiocarcinoma. Liver tissue was frequently examined with immunohistochemical staining for RECK, MMP2 and MMP9. The results of the control group implied that the expression of *RECK* was high but there was no sign of *MMP2* or *MMP9* expression. In group two, *RECK* expression was less intense when precancerous lesions had started to develop. When the cholangiocarcinoma was manifest, no RECK staining was observed at all in cancer cells, but intense RECK expression still prevailed in most surrounding hepatocytes. Positive staining of MMP2 and MMP9 was observed in both precancerous tissue and in tumor cells (18).

Pathophysiological Role of RECK

RECK is expressed in all normal tissues in rodents, as well in man (3). It has been demonstrated in tissue samples from various invasive human cancers that the *RECK* gene is either down-regulated or not expressed above detection level (4-7).

In one study, which aimed to demonstrate lack of RECK expression in invasive and metastatic breast cancer, significant results confirmed a decreased *RECK* expression as a negative survival factor for patients with breast cancer. Low expression levels of *RECK* were correlated with the occurrence of lymph node metastasis. However, the results were not significantly correlated with age, menopausal status or tumor size (6). Several other studies have also demonstrated similar results in liver, pancreas, breast, melanoma, fibrosarcoma, colon and lung malignancies and malignant glioma (3-7, 19-20)

One of the earliest examples of *RECK* down-regulation was how it correlated with aberrant activation of the *RAS* oncogene (21). This demonstrated a particularly important link to the intracellular mediation of mitogenic messages. The main cause of carcinogenesis is considered to be changes in the cellular genome that affect the expression or function of genes controlling cell growth and differentiation. The family of *RAS* genes is frequently mutated in human tumors. The RAS proteins resemble the G-proteins both functionally and structurally where they control adenylate cyclase. This implies that normal $p21^{RAS2}$ proteins are involved in the transduction of external stimuli that induce growth or cell differentiation (21). The connection between RAS and RECK was demonstrated by Takahashi *et al*. by detecting endogenous *RECK* mRNA in untransformed mouse NIH 3T3 cells, which was down-regulated in *Ras*-transformed cells (3). When *RAS* is turned on, the *RECK* gene is down-regulated leading to amplified secretion of MMP9, which contributes to the invasive capacity and morphological transformation of the cells. RAS signaling positively regulates MMP9 through multiple mechanisms, mainly through transcriptional activation, while RECK acts as a negative down-regulator of MMP9 (Figure 2) (3).

To metastasize, a tumor needs to possess the ability to induce angiogenesis and invasiveness. Tumor angiogenesis implies that cancer cells recruit blood vessels for their survival as the tumor enlarges. Invasiveness is by definition a tumor which escapes from its original site by penetrating the basal lamina and other ECM structures. This implies that if these functions can be inhibited, tumor growth will cease. The ECM provides a substantial framework upon which cells grow, migrate and differentiate, which is the main aim in the life of a tumor. The ECM consists primarily of collagen, proteoglycans and glycoproteins, for instance laminin and fibronectin. During growth and development, it is necessary for the ECM to constantly remodel; this process requires the extracellular proteases MMPs. They are necessary in wound healing, osteoporosis, rheumatoid arthritis and cancer. In addition, for metastasis, active MMPs are required for the tumor to break through the basal lamina and migrate. Three types of MMPs are potentially involved in the process of cancer, MMP9, MMP2 and MT1–MMP (14). It is known

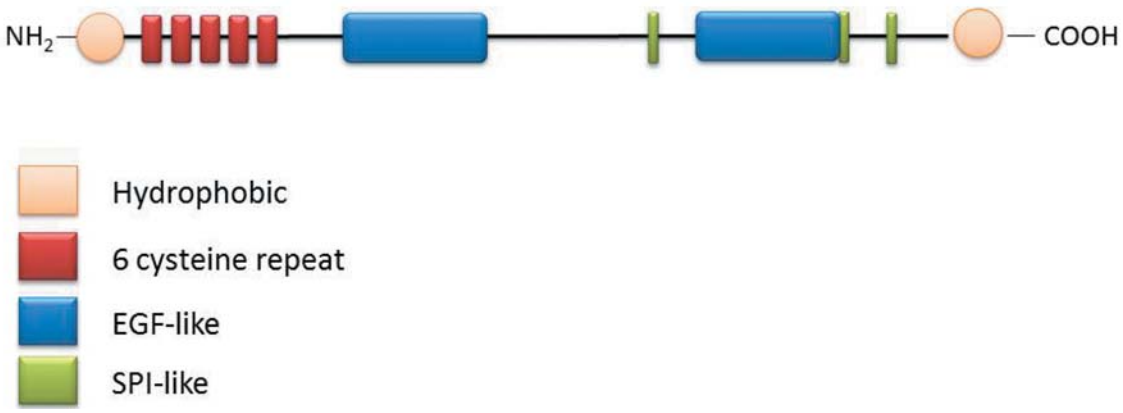

Figure 1. *The general structure of the RECK protein. Adapted from (3). EGF: Epidermal growth factor; SPI: Serine protease inhibitor.*

that RECK is a negative regulator of *MMP9* mRNA but not *MMP2* mRNA (22).

VEGF is a powerful inducer of angiogenesis, whereas it can either be soluble or bound to ECM structures or cell surfaces (23). Intratumoral microvessel density (IMVD) is a measurement of tumor angiogenesis which closely correlates with tumor progression and prognosis in a variety of malignant tumors. VEGF contributes to the IMVD value when activated (5). MMP9 is among several enzymes which are able to activate VEGF in tumor tissue, hence as *RECK* is down-regulated by oncogenes, *MMP9* is up-regulated and is then also able to contribute to angiogenesis (23). According to Takenaka *et al*. there is a significant inverse correlation between RECK and VEGF, which suggests that RECK suppresses angiogenesis induced by VEGF (5).

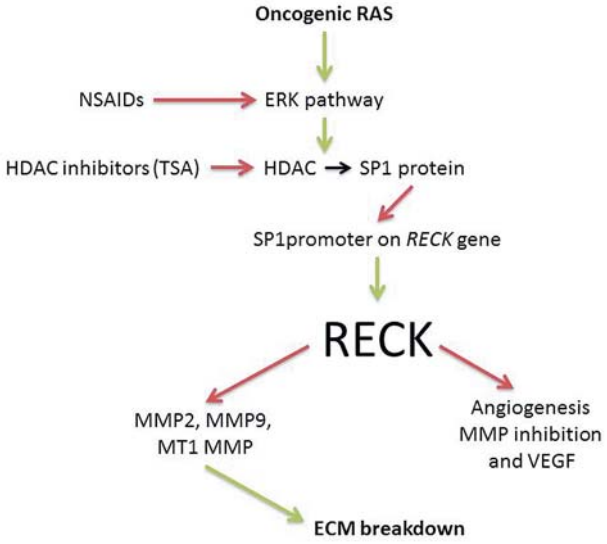

Figure 2. *Summary of RECK control. SP1 promoter site is affected by several different pathways, thereby inhibiting RECK gene. Oncogenic RAS is the primary activator. NSAIDs and TSA acts as inhibitors; they show potential for cancer therapy by reducing RECK down-regulation. The primary function of RECK is to inhibit ECM breakdown and angiogenesis. Adapted from (11). HDAC: Histone deacetylase; ERK: Extracellular signal-regulated kinase; MMP Matrix metalloproteinase; MT1: Metallothionein-1; VEGF: Vascular endothelial growth factor.*

Inhibition of MMPs

The family of MMPs are degradation enzymes of the ECM, and are endopeptidases that depend on Ca^{2+}, Zn^{2+} and neutral pH for activation (24). The MMP family consists of more than 20 members and they are divided into eight groups because of their shared functional domains, five of them are secreted and three are membrane-bound (8). They are synthesized as secreted or transmembrane pro-enzymes. Removal of an amino-terminal pro-peptide on the MMPs converts them into an active form. It is thought that the enzyme rests in latent form, while the propeptide is attached at the time of interaction of a cysteine residue. In the pro-peptide, the enzyme is maintained in an inactive, latent form by a specific interaction between cysteine and a zinc motif is the active site of the enzyme. If this interaction is disrupted, it will trigger a cysteine switch mechanism, which will activate the enzyme (24). The cysteine switch means that a cysteine blocks a zinc atom of the active site, which keeps the enzyme inactive. Conversely, the dissociation of cysteine from the zinc atom is considered as a switch that leads the enzyme to activation (25). The cleavage can be induced by other MMPs or by other proteases. Alternatively, they can be activated by chaotropic agents. Upon activation, the MMP can be inhibited by a family of TIMPs, or by small molecules with TIMP-like domains (24). TIMP1 -2, -3 and -

4 are the best studied inhibitors. They reversibly inhibit MMPs in a 1:1 stoichiometric fashion. They appear to be tissue-specific and differ in their ability to inhibit different types of MMPs (8). However, RECK is the only known membrane-bound inhibitor of MMPs (14).

MMPs are important in cancer research because of their correlation with high expression and activity of MMPs in almost every type of human and rodent cancer. They are also correlated with advanced tumor stage, increased invasion and metastasis and shortened survival. However, TIMP1 and -2 also have a connection with poor prognosis in cancer. High TIMP levels are equivalent to high levels of MMP, although expression favors MMPs and therefore high levels of TIMP can be associated with unfavorable tumor progression, but not cause it. Furthermore, TIMPs can also up-regulate VEGF secretion (8).

It has been demonstrated that overexpression of *RECK* reduces the level of MMP9 mRNA. *In vitro*, RECK and both MMP2 and MMP9 were correlated. Overexpression of *RECK* lowered the level of *MMP9* mRNA but the level of *MMP2* mRNA stayed unaffected. However, MMP9 recovered when *RECK* expression was silenced, leaving levels of MMP2 unaffected (22). However, when the effect of RECK expression on *MMP9* promoter activity was examined, the promoter region was found to be located at −700 to −400 bp and the results acknowledged that *MMP9* promoter region is exposed by RECK-mediated suppression (22).

The tight regulation of MMP genes is important for tissue breakdown and reconstruction balance. If overexpressed, several other pathological conditions arise as a consequence of connective tissue destruction. These include diseases such as arthritis and periodontitis (26). Recently, the role of RECK became highlighted in the progression of unstable coronary atherosclerotic plaques. Since microRNA-21 has been shown to support the progression of atherosclerosis, it was also found that it acts *via* MMP9 and is augmented by the down-regulation of RECK (26).

Inhibition of Tumor Angiogenesis

Cells have two ways of recruiting new blood vessels: either by angiogenesis or vasculogenesis. The two approaches differ. Angiogenesis is the process by which new vessels grow from existing endothelial lined vessels and is therefore an invasive process which requires proteolysis of ECM, proliferation, synthesis of new matrix components and migration of endothelial cells. The angiogenic response is important in most pathological conditions as *e.g.* inflammation, hypoxia and wound healing. Angiogenesis is also important during tumor growth and metastasis. Meanwhile, vasculogenesis implies that endothelial cells develop by proliferation from existing stem cells. This requires the presence of stem cells, in particular angioblasts, which differentiate and migrate in response to signals. This occurs during fetal development where angiogenesis is absent (27).

The VEGF family of proteins are normally expressed in endothelial cells where they are either freely soluble or bound to cell surfaces and ECM due to their heparin-binding properties (23). They are the main inducers of angiogenesis and are involved in several functions in the body such as bone formation, hematopoiesis, wound-healing and development, *i.e.* every process in which angiogenesis has a primary role. VEGF is also produced by other cells connected to the vascular system, but it is also known that VEGF is produced by tumor cells (28). VEGF was induced by MMP9 in a study on multistage pancreatic cancer. MMP9 proved to be the only proteinase to increase the release of VEGF *in vitro* and a selective inhibitor of MMP9 was the most effective blockade of the initial angiogenic switch (23). Along with this, it is known that MMPs have more tasks than ECM breakdown to form new vessels; they can also promote angiogenesis by regulating endothelial cell attachment, proliferation, growth and migration. Other studies even show that MMPs can generate or release angiogenic inhibitors such as angiostatin from the ECM. Tumor macrophages can secrete MMP9, which can indirectly affect endothelial cell behavior by releasing pro-angiogenic factors and others (27). This correlation between MMP9 and VEGF has been demonstrated in canine lymphoma. The results were based on a comparison between B-cell and T-cell lymphoma *in vivo* by immunocytochemical staining to specifically detect VEGFA, which is the most common VEGF family member. It was shown that high expression of *VEGFA* mRNA and protein were detected in both B-cell and T-cell lymphoma. The levels of *VEGFA* mRNA were correlated with the levels of MMP9 (29). In canine mast cell tumors, the result was similar, where the mast cells released high levels of *VEGFA* mRNA, as well as protein, equivalent to expression of MMP9 production (30).

Correlation between *RECK* expression and tumor angiogenesis showed that VEGF was not detected in normal colorectal mucosa. However, it was present in carcinoma cells, mainly in their cytoplasm or membranes. A total of 26.4% of the specimens were classified as expressing high levels of VEGF and an inverse correlation between *RECK* and *VEGF* expression was clearly significant. A significant inverse correlation between RECK and microvessel density was also found (4). Microvessel density is a measure of the number of vessels per high-power (microscope) field and reflects the intercapillary distance. The net balance between stimulatory and inhibitory angiogenic factors, which ultimately results in local formation of vessels in microregions, affects the intercapillary distance. Similar effects were achieved by non-angiogenic factors, including oxygen and nutrient consumption (31).

The possibility that RECK suppresses tumor angiogenesis induced by VEGF was also investigated in a study of non-small cell lung cancer. The study found *RECK* expression inversely correlated with IMVD and the effects of RECK where shown in tumors expressing VEGF at higher levels. Consequently when *RECK* expression was weak in tumor cells, the mean IMVD increased, followed by an increased VEGF score and *vice versa* (5).

Recent data showed limited vascularization in tumors, with high RECK expression. Such tumors had few large blood vessels where only the cells surrounding them survived. This indicated that a high level of *RECK* expression limits the angiogenic function of the tumor, which leads to decreased tumor growth (14).

Concluding Remarks

Down-regulation of the *RECK* gene is directly correlated with tumor invasion, metastasis and angiogenesis (11). The *RECK* gene is a relatively recent discovery and has become a central topic in the discussion of new diagnostic biomarkers for detecting early stages of cancer and of the possibility of developing indicators of prolonged survival for patients with cancer (6). The RECK protein does not have a cytotoxic effect on tumor cells (6). Expression of *RECK* simply inhibits tumor growth by regulating ECM breakdown and inhibition of the formation of new blood vessels. When these processes are halted, the tumor is unable to break through the basal lamina and to spread to other places in the body. In other words, the metastatic process is blocked (14). Normally the RECK protein regulates MMP activity, which is important during wound healing and, for example, osteoporosis. When RECK acts efficiently, it inhibits MMPs so that ECM breakdown is disrupted. When RECK is absent, the breakdown of ECM is totally unopposed, facilitating tumor growth and metastasis (3). The *RECK* expression pattern is conserved in several species and the most important and evaluated are canine, mouse and human cell lines. The discovery of *RECK* in several species suggest that the gene has a fundamental and phenotypically-conserved biological role (13, 32).

It is known that RECK inhibits MMPs, especially MMP9 which is an important ECM dilapidator (22). MMP9 also has a close relationship with another powerful inducer of angiogenesis, VEGF (29). In this way, RECK expression is important because it plays a role in constructive as well as destructive processes. A metastatic tumor requires *de novo* vessel formation to be able to continue to grow, as well as efficient ECM breakdown. If both VEGF and MMPs are inhibited by RECK expression, it implies that RECK plays a highly significant role in biological malignancy. One recent study highlighted the possibility of transfecting cells with dsRNA complementary to the promoter of the *RECK* gene (33).

There are still unsolved issues considering the role of RECK, especially the exact correlation between VEGF function and RECK expression. Moreover, further *in vivo* studies are required for a better understanding of the multi-functional facets of the gene. Published studies suggest that RECK is a potential biomarker for early cancer detection and which may well be used for monitoring cancer treatment in the future.

References

1 Lemmon MA and Schlessinger J: Cell signalling by receptor tyrosine kinases. Cell *141*: 1117-1134, 2010.

2 Hanahan D and Weinberg RA: Hallmarks of cancer. Cell *144*: 646-674, 2011.

3 Takahashi C, Zeqi Sheng Z, Thomas P: Horan TP, Hitoshi Kitayama, H Masatoshi Maki M, Kiyotaka Hitomi K and Yasuyuki Kitaura Y: Regulation of Matrix Metalloproteinase-9 and Inhibition of Tumor Invasion by the Membrane-Anchored Glycoprotein RECK. Proc Natl Acad Sci (USA) *95*: 13221-13226, 1998.

4 Takeuchi T, Hisanaga M, Nagao M, Ikeda N, Fujii H, Koyama F, Mukogawa T, Matsumoto H, Kondo S and Takahashi C: The Membrane-Anchored Matrix Metalloproteinase (MMP) Regulator RECK in Combination with MMP-9 Serves as an Informative Prognostic Indicator for Colorectal Cancer. Clin Cancer Res *10*: 5572-5579, 2004.

5 Takenaka K, Ishikawa S, Kawano Y, Yanagihara K, Miyahara R, Otake Y and Morioka Y: Expression of a Novel Matrix Metalloproteinase Regulator, RECK, and Its Clinical Significance in Resected Non-Small Cell Lung Cancer. Eur J Cancer *40*: 1617-1623, 2004.

6 Zhang G, Cheng S, Zhang G, Ma W, Liu Y, Zhao R, Zhang Q and Pang D: Low Expression of RECK Indicates a Shorter Survival for Patients with Invasive Breast Cancer. Cancer Sci *103*: 1084-1089, 2012.

7 Masui T, Doi R, Koshiba T, Fujimoto K, Tsuji S, Nakajima S, Koizumi M, Toyoda E, Tulachan S and Ito D: RECK Expression in Pancreatic Cancer Its Correlation with Lower Invasiveness and Better Prognosis.Clin Cancer Res *9*: 1779-1784, 2003.

8 Egeblad M and Werb Z: New Functions for the Matrix Metalloproteinases in Cancer Progression. Nature Rev Cancer *2*: 161-174. 2002.

9 Long NK, Kato K, Yamashita T, Makita H, Toida M, Hatakeyama D, Hara A, Mori H and Shibata T: Hypermethylation of the RECK Gene Predicts Poor Prognosis in Oral Squamous Cell Carcinomas. Oral Oncol *44*: 1052-1058, 2008.

10 Tian M and Kamoun S: A Two Disulfide Bridge Kazal Domain from Phytophthora Exhibits Stable Inhibitory Activity against Serine Proteases of the Subtilisin Family. BMC Biochem *6*: 15, 2005.

11 Clark JCM, David M. Thomas DM, Choong PFM and Dass CR: RECK–a Newly Discovered Inhibitor of Metastasis with Prognostic Significance in Multiple Forms of Cancer. Cancer Metast Rev *26*: 675-683, 2007.

12 Wilson J: Cloning and Analysis of RECK during Early Xenopus Laevis Development. University of Western Ontario – Electronic Thesis and Dissertation Repository, November 30, 2012.

13 NCBI (2014-03-09). HomoloGene: 9622. http://www.ncbi.nlm.nih.gov/homologene/9622Last accessed 2014-03-12
14 Oh J, Takahashi R, Kondo S, Mizoguchi A, Adachi E, Sasahara RM and Nishimura S: The Membrane-Anchored MMP Inhibitor RECK Is a Key Regulator of Extracellular Matrix Integrity and Angiogenesis. Cell *107*: 789-800, 2001.
15 Noda M, Oh J, Takahashi R, Kondo S, Kitayama H and Takahashi C: RECK, a novel suppressor of malignancy linking oncogenic signaling to extracellular matrix remodeling. Cancer Metastat Rev *22*: 167-175, 2003.
16 Peng X, Wu W, Zhu B, Sun Z, Ji L, Ruan Y, Zhou M, Zhou L, and Gu J: Activation of farnesoid receptor induces RECK expression in mouse liver. Biochem Biophys Res Comm *443*: 211-216. 2014.
17 Zhang X, Healy C and Nothnick WB: Estrogen suppresses expression of the matrix metalloproteinase inhibitor reversion inducing cystein rich protein with Kazal motifs (RECK) within the mouse uterus. Endocrine *42*: 97-106, 2012.
18 Namwat, N., Puetkasichonpasutha J, Loilome W, Yongvanit P, Techasen A, Puapairoj A, Sripa B, Tassaneeyakul T, Khuntikeo N and Wongkham S: Downregulation of Reversion-Inducing-Cysteine-Rich Protein with Kazal Motifs (RECK) Is Associated with Enhanced Expression of Matrix Metalloproteinases and Cholangiocarcinoma Metastases. J Gastroenterology *46*: 664-675, 2010.
19 Chung TT, Yeh CB, Li YC, Su SC, Chien MH, Yang SF and Hsieh YH: Effect of RECK Gene Polymorphisms on Hepatocellular Carcinoma Susceptibility and Clinicopathologic Features PLoS ONE *7*: e33517. 2012.
20 Chen Y and Tseng SH: The potential of RECK inducers as antitumour agents for glioma. Anticancer Res *32*: 2991-2998, 2012.
21 Bos JL: Ras Oncogenes in Human Cancer: A Review. Cancer Res *49*: 4682-4689, 1989.
22 Takagi S, Simizu S and Osada H: RECK Negatively Regulates Matrix Metalloproteinase-9 Transcription. Cancer Res *69*: 1502-1508, 2009.
23 Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K and Tanzawa K: Matrix Metalloproteinase-9 Triggers the Angiogenic Switch during Carcinogenesis. Nature Cell Biol *2*: 737-744, 2000.
24 Vu TH: Matrix Metalloproteinases: Effectors of Development and Normal Physiology. Genes Developm *14*: 2123-2133, 2000.
25 Chakraborti S, Malay Manda M, Sudip Das S, Amritlal Mandal A, and Tapati Chakraborti T; Regulation of Matrix Metalloproteinases: An Overview. Molecular and Cell Biochem *253*: 269-285, 2003.
26 Fan X, Wang E, Wang X, Cong X and Chen X: MicroRNA-21 is a unique signature associated with coronary plaque instability in humans by regulating matrix metalloproteinase 9 *via* reversion inducing cysteine rich protein with Kazal motifs. Exp Mol Pathol *96*: 242-249, 2014.
27 Stetler-Stevenson WG: Matrix Metalloproteinases in Angiogenesis: A Moving Target for Therapeutic Intervention. J Clin Investig *103*: 1237-1241, 1999.
28 Tammela TB, Enholm B, Alitalo K and Paavonen K: The Biology of Vascular Endothelial Growth Factors. Cardiovascular Res *65*: 550-563, 2004.
29 Aricò A, Giantin M, Gelain ME, Riondato F, Comazzi S, Rütgen BC, Essler SE, Mauro Dacasto M, Castagnaro M and Aresu L; The role of vascular endothelial growth factor and matrix metalloproteinases in canine lymphoma: *in vivo* and *in vitro* study. BMC Vet Res *9*: 94, 2013.
30 Giantin M, Aresu L, Benali S, Aricò A, Morello EM, Martano M and Vascellari M: Expression of Matrix Metalloproteinases, Tissue Inhibitors of Metalloproteinases and Vascular Endothelial Growth Factor in Canine Mast Cell Tumours. J Comp Path *147*: 419-429, 2012.
31 Hlatky L, Hahnfeldt P and Folkman J: Clinical Application of Antiangiogenic Therapy: Microvessel Density, What It Does and Doesn't Tell Us. J Natl Cancer Inst *94*: 883-893, 2002.
32 Takagi S, Kitamura T, Hosaka Y, Ohsaki T, Bosnakovski D, Kadosawa T, Okumura M and Fujinaga T: Molecular Cloning of Canine Membrane-Anchored Inhibitor of Matrix Metalloproteinase, RECK. J Vet Med Sci 67: 385-391, 2005.
33 Sakurai F, Nanjo Y, Okamoto S, Tachibana M and Mizuguchi H: Up-regulation of RECK gene expression by small double stranded RNA targeting the promoter region. Cancer Gene Ther *21*: 164-170, 2014.

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