Sveriges lantbruksuniversitet
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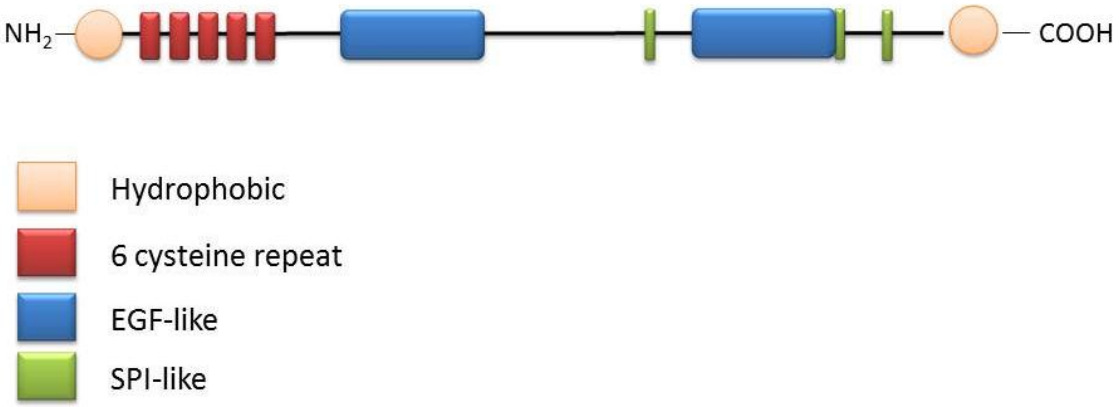
The RECK gene and invasive cancer development

The significance of RECK in angiogenesis and inhibition of matrix metalloproteinases

Michelle Alexius Lindgren



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RECK-genen och invasiv canceruppkomst

Betydelsen av RECK vid angiogenes och hämning av matrix metalloproteinaser.

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CONTENT LIST

SAMMANFATTNING

RECK genen är en relativt nyupptäckt gen som blivit högintressant inom cancerforskningen. Det är främst inom den humana medicinen som genen studerats för att eventuellt kunna registrera cancerns invasiva egenskaper. Uppreglerad *RECK* innebär ofta en avsevärt förlängd livstid hos patienter med allvarliga former av elakartad cancer

Normalt uttrycks *RECK* i alla kroppens celler och har då en viktig roll i balansen mellan nedbrytande och uppbyggande funktioner av extracellulära matrix (ECM). RECK-proteinet är ett membranbundet glykoprotein som inhiberar matrix metalloproteinaser vilka har till uppgift att bryta ner ECM. Man har också hittat ett samband mellan *RECK*-genens uttryck och nybildning av kärl, närmare bestämt via VEGF som är en viktig och kraftig stimulant av angiogenes. Nedregleringen av *RECK* orsakas av *Ras*-onkogenen som annars också är en vanlig orsak till tumörutveckling i tidigt skede.

För att en tumör skall utvecklas och få egenskaper som klassificerar den som malign, blir nedbrytning av ECM och mobilisering nya blodkärl steg på vägen. Om tumören hindras i dessa funktioner kommer den inte kunna utvecklas och växa som den vill, därför ser man RECK som en potentiell hämmare men även som en markör för att tidigt kunna bedöma den enskilda tumörens biogena malignitet och därmed hur sjukdomsförloppet för en cancerpatient blir.

SUMMARY

The *RECK* gene is a relatively new discovered gene with important implications for cancer research. The research has been primarily concentrated on the human gene with the ultimate aim to identify the invasive characteristics. Up regulated *RECK* is linked to significantly prolonged survival rates in patients with severe forms of malignancies.

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. The RECK protein is a membrane-bound glycoprotein that inhibit matrix metalloproteinases which has 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 VEGF which is an important and powerful inducer of angiogenesis. Research has shown that the downregulation of *RECK* is caused by the *Ras* oncogene, which otherwise also is a common cause of tumor development in the early stage.

For a tumor to progress and gain characteristics that classifies it as malignant, the degradation of ECM and mobilization of new blood vessels are essential functions. If the tumor is inhibited with respect to these functions the tumor will cease to grow. RECK is therefore a potential inhibitor but also a prognostic marker available at early clinical stages.

INTRODUCTION

The behavior of normal cells is regulated by a variety of signals. Broadly speaking signals can be either external or internal. However when an external signal gets in contact with the cell, the transcription of genes that encodes proteins necessary for the intracellular mediation of the specific message is elicited. 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, the cell ceases to respond to these regulatory signals. This is mainly because that genes responsible for growth inhibition or to take the cell into apoptosis, does not operate in accordance with control procedures. Early genes can either be up regulated or down regulated and then result in dysfunction leading to overexpression or silencing of other regulatory genes. Gene silencing is often the result of altered DNA-methylation, i.e. when a methyl group is connected to a CG base motif and thereby creating a non-readable frame. 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 unwarranted mishaps occur, transformed cells can grow and divide in an uncontrolled manner, invading normal tissue and leading to metastasis throughout the body.

Recently the *RECK* gene has been highlighted because of its correlation with metastasis and invasiveness. 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 controlling the activity of remodeling enzymes as e.g. matrix metalloproteinases (MMPs), especially MMP-9. This quality is important in, for example wound healing and other physiological features.

It was discovered that the level of *RECK* expression was correlated with the biological phenotype of different cancer types. *RECK* was found to be down regulated in tumors which were invasive and metastatic. Several tumors has been tested for their RECK expression levels and there has been a clear correlation between RECK expression and the biological malignancy of liver-, pancreas-, breast-, melanoma-, fibrosarcoma-, colon- and lung tissue. *RECK* acts as a suppressor gene which inhibits angiogenesis, invasion and metastasis. However, multivariate analysis showed that the methylation status of the RECK gene was the only independent prognostic factor affecting overall survival of cancer patients ($p = 0.037$). This suggests that *RECK* is a promising biomarker in the early detection of cancer. (Long et al., 2008)

MATERIAL AND METHODS

This is a literature study where the main material is referred to the webpage pubmed.com. The search was mainly correlated to words such as “RECK”, “tumor”, “matrix metalloproteinases ”,“ MMP ”,“ angiogenesis ”,“ metastasis ”,“ gene expression ”,“ cancer” and “VEGF”.

LITTERATURE REVIEW

Gene structure

RECK is named after its structural features and its activity, Reversion-inducing Cysteine-rich protein with Kazal motifs. (Takahashi et al., 1998) 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 (Tian and Kamoun, 2005). 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 matrix metalloproteinase-9. 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 (Takahashi et al., 1998). 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 the p16INK4a which plays a particularly distinct role in melanoma. (Clark et al., 2007)

The human *RECK* gene spans over 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 lie four of these SNPs which increase the opportunity of disease implication. Polymorphisms lead to morbid function in the RECK protein structure (Clark et al., 2007) 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. (Willson, 2012)

The RECK gene is also conserved in chimpanzee, Rhesus monkey, cow, rat, chicken, fruit fly, and mosquito. (NCBI, 2014)

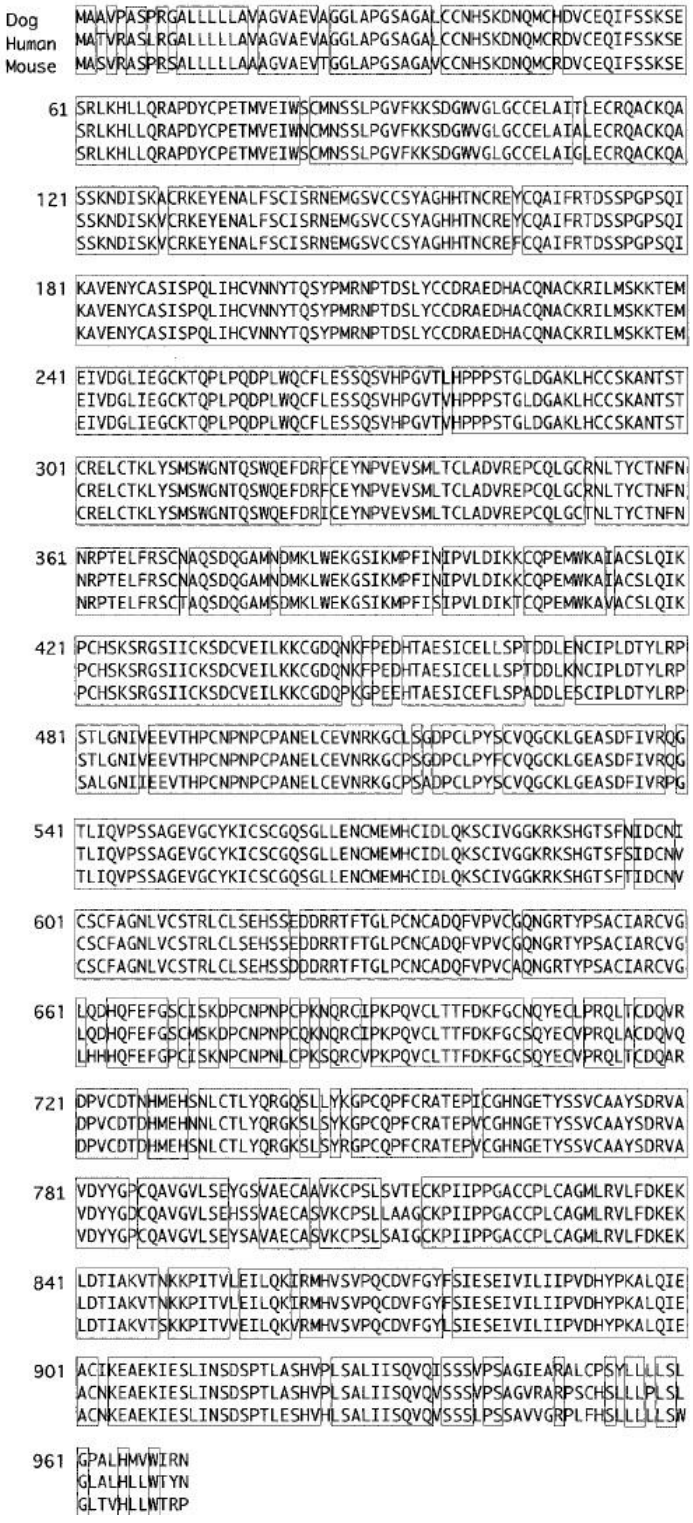

Fig. 1 *Comparison of amino acids between canine, mouse and human, of the RECK sequence. Identical aminoacids are in boxes. (Takagi et al., 2005)*

Protein structure

The RECK protein consists of 971 amino acids and according to Takahashi et al., 1998 shows that both the human and mouse cDNA share 93.0% identity with each other. The protein is cysteine rich (9%) and it includes hydrophobic regions in both ends, 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 serineprotease inhibitor-like (SPI) domains. The initial parts of the middle domain correspond to the Kazal motif while the second and third do not. (Takahashi et al., 1998) These SPIs are believed to inhibit the protease activity, either by physical 'trapping' or by 'reversible tight binding'. SPI;s most probably play an important role in the inhibition of MMP. (Clark et al., 2007) Also there are two regions with epidermal growth factor (EGF)-like repeats in the middle domain space of the protein. RECK is named after these structural features and its activity, Reversion-inducing Cysteine-rich protein with Kazal motifs. (Takahashi et al., 1998)

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 and as well containing several glycosylation sites at asparagine (Asn) residues. These sites are essential for obtaining proper interaction with MMP-9 and MMP-2 which is the core function of the RECK protein. (Clark et al., 2007)

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

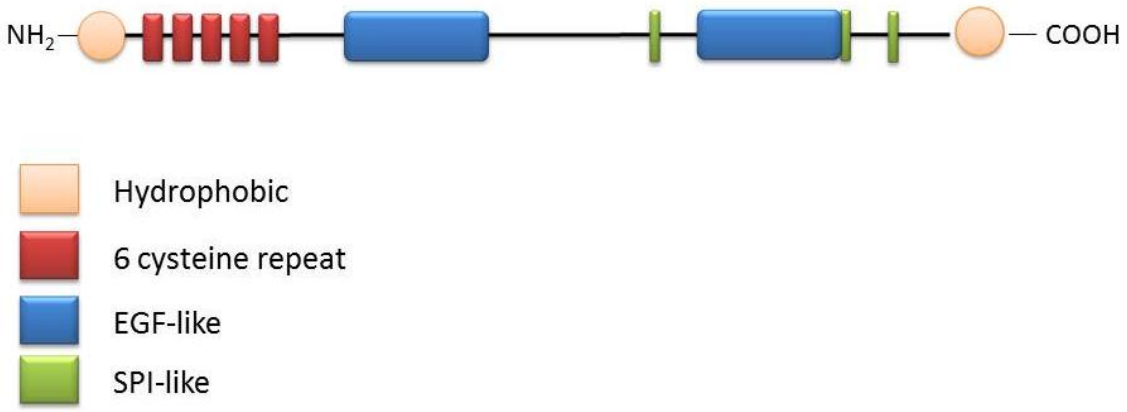

Fig. 2 *The general structure of the RECK protein. Adapted from Takahashi et al., 1998*

Physiological role of RECK

The primary function of RECK is to serve as an important mediator of tissue remodeling. RECK inhibits matrix metalloproteinase (MMP-2 and MMP-9) and MT1-MMP post-transcriptionally. MMP-2 and MMP-9 break down extra-cellular matrix (ECM) both under normal conditions and during pathological conditions. Normally *RECK* is expressed in all human and mammalian organ cells including mesenchymal tissues and in vascular smooth muscle cells. (Oh et al., 2001)

In a study by Takahashi et al., 1998 it was confirmed that activated *Ras*-oncogenes suppress the *RECK* promoter. Down-regulation of *RECK* mRNA was also induced by a variety of other oncogenes, such as *v-fos, c-myc, v-src, v-fms, v-fes* and *v-mos*. (Takahashi et al., 1998) Dramatic morphological alternations developed as a result of mutated *Ras*-genes, which occurs often in many kinds of tumors. (Sasahara et al., 1999)

Oh et al., 2001 created KO-mice with a disrupted *RECK* gene in order to understand the physiological significance. 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 embryo 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 suggest a deficient blood maturation and not vasculogenesis.

RECK expression			
Organ			
Heart			(-)
Brain			-
Placenta		+	
Lung	++		
Liver			(-)
Skeletal muscle		+	
Kidney		+	
Pancreas			-
Spleen		+	
Thymus		+	
Prostate		+	
Testis	++		
Ovary	++		
Small intestine	++		
Colon mucosa	++		
Leukocyte			(-)

Table. 1.

Detection of RECK mRNA in human organs, done by Northern blot hybridization. Adapted from Takahashi et al., 1998

The physiological role of RECK in animal experiments

To be able to understand the main functions of RECK there has been several studies made in vivo. (Oh et al., 2001) (Namwat et al., 2010) 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, 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 in 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 differ from embryos with a functional *RECK* since they have 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. (Oh et al., 2001) The difference in vasculogenesis and

angiogenesis will be discussed later on.

A study on Syrian golden hamsters was performed by Namwat et al., 2010, where the hamsters were divided into two groups. Group 1 remained untreated and group 2 was infected with virus to develop cholangiocarcinoma. Liver tissue was frequently examined with immunohistochemical staining for RECK, MMP-2 and MMP-9. The results of the control group implied that the expression of *RECK* was high but there was no sign of *MMP-2* or *MMP-9* 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 MMP-2 and MMP-9 was observed in both precancerous tissue and in tumor cells. (Namwat et al., 2010)

Pathophysiological role of RECK

RECK is expressed in all normal tissues in rodents as well in man. (Takahashi et al., 1998) 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. (Takeuchi et al., 2004) (Takenaka et al., 2004) (Masui et al., 2003) (Zhang et al., 2012)

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 breast cancer. Low expression levels of RECK were correlated with the occurrence of lymph node metastasis. However, the results were not significantly for correlated with age, menopausal status or tumor size. (Zhang et al., 2012)

Several other studies have also demonstrated similar results in liver, pancreas, breast, melanoma, fibrosarcoma, colon and lung malignancies. (Chung et al., 2012) (Zhang et al., 2012) (Takahashi et al., 1998) (Masui et al., 2003) (Takeuchi et al., 2004) (Takenaka et al., 2004)

One of the earliest examples of *RECK* down regulation was how it correlated with aberrant activation of the *Ras* oncogene. 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 them control adenylate cyclase. This implies that normal p21ras2 proteins are involved in the transduction of external stimuli that induce growth or cell differentiation. (Bos, 1989) The connection between Ras and RECK was demonstrated by Takahashi et al., 1998 by detecting endogenous *RECK* mRNA in untransformed mouse NIH 3T3 cells, which was down-regulated in Ras-transformed cells. When Ras is turned on, the *RECK* gene is down-regulated leading to amplified secretion of MMP-9, which contributes to the invasive capacity and morphological transformation of the cells. Ras signaling positively regulates MMP-9 through multiple mechanisms, mainly through transcriptional activation, while RECK acts as a negative down-regulator of MMP-9. (Takahashi et al., 1998)

To metastasize, a tumor needs to possess the ability to induce angiogenesis and invasiveness. Tumor angiogenesis implies that cancer cells are recruiting 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 extracellular matrix structures. This implies that if these functions can be inhibited, the tumor growth will cease. Extracellular matrix supplies a substantial framework upon which cells grow, migrate and differentiate which is the main aim in the life of a tumor. Extracellular matrix (ECM) consists primarily of collagen, proteoglycans and glycoproteins, for instance laminin and fibronectin. During growth and development of the body, it's necessary for the ECM to constant remodel, this process requires extracellular proteases named matrix metalloproteinases (MMPs). They are necessary in wound healing, osteoporosis, rheumatoid arthritis and cancer. In addition it's also required for the metastatic process of the tumor to have active MMPs for the tumor to break through the basal lamina and migrate. Three types of MMPs are potentially involved in the process of cancer, MMP-9, MMP-2 and MT1-MMP. (Oh et al., 2001) It is known that RECK is a negative regulator of MMP-9 mRNA but not MMP-2 mRNA. (Takagi et al., 2009)

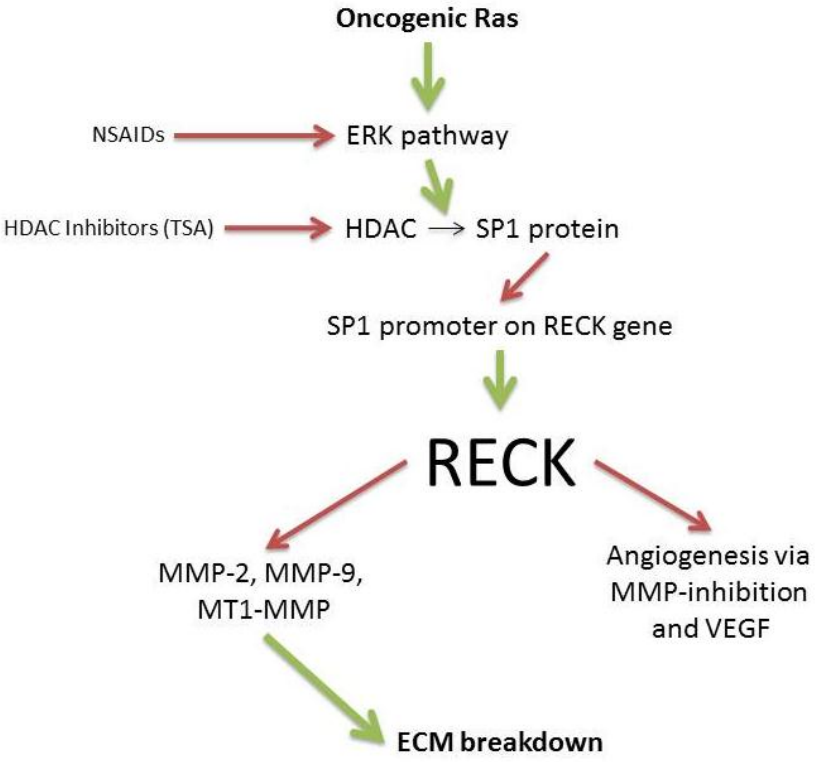

Fig. 3
Summary of RECK controls. Sp1 promoter site is affected by several different pathways and thereby inhibiting RECK gene. Oncogenic Ras is the primarily activator. NSAID and TSA acts as inhibitors, they show potential for cancer therapy by lowering RECK down regulation. The primary function of RECK is to inhibit ECM breakdown and angiogenesis. Adapted from Clark et al., 2007.

Vascular endothelial growth factor (VEGF) is a powerful inducer of angiogenesis whereas it can either be soluble or bound to ECM structures or cell surfaces. (Bergers et al., 2000) 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 is contributing to the IMVD value when activated. (Takenaka et al., 2004) MMP-9 is among several enzymes which are able to activate VEGF in tumor tissue, so as *RECK* gets down-regulated by oncogenes; *MMP-9* is up-regulated and is then able to contribute to angiogenesis as well. (Bergers el al., 2000) According to Takenaka et al., 2004 there is a significant inverse correlation between RECK and VEGF which suggests that RECK suppresses angiogenesis induced by VEGF.

Inhibition of Matrix Metalloproteinases (MMPs)

The family of matrix metalloproteinases (MMPs) is degradation enzymes of the extracellular matrix (ECM), they're endopeptidases that depend on Ca^{2+}, Zn^{2+} and neutral pH for activation. (Vu, 2000) The MMP family consists of >20 members and they are divided in to eight groups because of their shared functional domains, five of them are secreted and three are membrane-bound. (Egeblad and Werb, 2002) By removal of an amino-terminal propeptide on the MMPs they precede in an active form. Before this happens they are synthesized as secreted or transmembrane proenzymes. It is thought that the enzyme rests in latent form while the propeptide is attached in time of interaction of a cysteine residue. In the propeptide, 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 gets disrupted it will trigger a cysteine switch mechanism, which will activate the enzyme. (Vu, 2000) The cysteine switch means that a cysteine (Cys), blocks a zinc atom of the active site which keeps the enzyme inactive. Conversely the dissociation of Cys from the zinc atom is considered as a switch that leads the enzyme to activation. (Chakraborti et al., 2003) 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 tissue inhibitors of metalloproteinases, TIMPs, or by small molecules with TIMP-like domains. (Vu, 2000) TIMP 1 -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. (Egeblad and Werb, 2002) However, RECK is the only hither to known membrane-bound inhibitor of MMPs. (Oh et al., 2001) 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. But, TIMP-1 and -2 also have a connection with poor prognosis in cancer. High TIMP levels are equivalent to high levels of MMP, though the expression is flavoring the MMPs and therefore high levels of TIMP can be associated with unfavorable tumor progression, but not cause it. Furthermore, TIMPs can also up-regulate vascular endothelial growth factor (VEGF) secretion. (Egeblad and Werb, 2002)

The tight regulation of MMP genes is important for tissue break-down 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. (Matrisian, 1994)

It has been demonstrated that overexpression of *RECK* decreases the level of MMP-9 mRNA. In vitro, RECK/MMP-9 and RECK/MMP-2 where correlated. Overexpression of *RECK* lowered the levels of MMP-9 mRNA but the levels of MMP-2 mRNA stayed unaffected. But,

MMP-9 recovered when *RECK* expression was silenced, leaving levels of MMP-2 unaffected. (Takagi et al., 2009) However, when the effect of *RECK* expression on *MMP-9* promoter activity was examined the promoter region was found to be located at -700 to -400 bp and the results acknowledged that *MMP-9* promoter region is exposed by RECK-mediated suppression. (Takagi et al., 2009)

Inhibition of tumor angiogenesis

Cells have two ways of to recruiting new blood vessels: either angiogenesis or vasculogenesis. The two approaches differ and angiogenesis is the process by which new vessels grow from existing endothelial lined vessels. Angiogenesis is an invasive process which requires proteolysis of extracellular matrix, 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. But, the process of recruiting blood vessels, angiogenesis, is also important during tumor growth and metastasis. Meanwhile, vasculogenesis implicates that endothelial cells develops 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. (Stetler-Stevenson, 1999)

VEGF or vascular endothelial growth factor is a family of proteins normally expressed in endothelial cells where they could either be freely soluble or bound to cell surfaces and ECM due to their heparin-binding properties. (Bergers et al., 2000) 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 prime 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. (Tammela et al., 2005) VEGF was induced by MMP-9 in a study on multistage pancreatic cancer. MMP-9 proved to be the only proteinase to increase the release of VEGF in vitro and a selective inhibitor of MMP-9 was the most effective blockade of the initial angiogenic switch. (Bergers et al., 2000) Along with this, it's 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. Later studies even show that MMP could generate or release angiogenic inhibitors such as angiostatin from the ECM. Tumor macrophages can secrete MMP-9 which can indirectly affect endothelial cell behavior by releasing proangiogenic factors and others. (Stetler-Stevenson, 1999) This correlation between MMP-9 and VEGF was demonstrated in canine lymphoma. The results were based on a comparison between B-cells and T-cells lymphoma in vivo by immunocytochemistry staining to specifically detect VEGF-A, which is the most common vascular growth factor in the VEGF-family. It was shown that high expression of mRNA and protein VEGF-A were detected in both B-cells and T-cells lymphoma. Also, the levels of VEGF-A mRNA was correlated with the levels of MMP-9. (Aricò et al., 2013) In canine mast cell tumors the result was similar, where the mast cells released high levels of VEGF-A mRNA as well as protein, equivalent to expression of MMP-9 production. (Giantin et al., 2012)

Correlation between *RECK* expression and tumor angiogenesis showed that they could not detect VEGF could not be detected in normal colorectal mucosa. However it could be detected in carcinoma cells, mainly in their cytoplasm or membranes. 26.4% of the specimens were classified as expressing high levels of VEGF and the result of an inverse correlation between *RECK* and *VEGF* expression was clearly significant. A significant relationship between RECK and microvessel density (MVD) was also found as an inverse correlation.

(Takeuchi et al., 2004) Microvessel density is a measure of the number of vessels per high-power (microscope) field and reflects the intercapillary distance. The net balance between stimulating and inhibitory angiogenic factors which ultimately result in local formation of vessels in microregion affects the intercapillary distance. Similar effects could be achieved by non angiogenic factors including oxygen and nutrient consumption rates. (Hlatky et al., 2002)

The possibility that RECK suppresses the tumor angiogenesis induced by VEGF was also observed in a study of non-small cell lung cancer. The results in the study presented inversely correlated *RECK* expression with IMVD and the effects of RECK where shown in tumors expressing VEGF in higher levels. Consequently when *RECK* expression was weak in the tumor cells, the mean IMVD increased followed by an increased VEGF-score and so in the other way. (Takenaka et al., 2004)

Oh et al. presented data that showed limited vascularization in very large *RECK* expression tumors, to a few large blood vessels where only the cells surrounding them survived. This indicated that levels of high *RECK* expression limits the angiogenic function of the tumor which leads to decreased tumor growth. (Oh et al., 2001)

DISCUSSION

Down regulation of the *RECK* gene is directly correlated with tumor invasion, metastasis and angiogenesis. (Clark et al., 2007) The *RECK* gene is a relatively recent discovery and has become a central topic in the discussion of new diagnostic biomarkers detecting early stages of cancer and the possibility of developing prolonged survival indicators for cancer patients. (Zhang et al., 2012) The RECK protein does not have a cytotoxic effect on tumor cells.(Takenaka et al., 2004) Expression of *RECK* simply inhibits the tumor growth by regulating extra cellular matrix breakdown and the 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. (Oh et al., 2001) Normally the RECK protein regulates matrix metalloproteinase activity which is important during wound healing and for example osteoporosis. When RECK is acting efficiently, it is inhibiting MMPs so that the ECM breakdown is disrupted. When RECK is absent the breakdown of ECM is totally unopposed and this facilitates tumor growth and metastasis. (Takahashi et al., 1998) The *RECK* expression pattern is conserved in several species and the most important and evaluated is 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. (NCBI, 2014)

It is known that RECK inhibits MMPs, especially MMP-9 which is an important ECM dilapidator. (Takagi et al., 2009) MMP-9 does also have a close relation to another powerful inducer of angiogenesis, VEGF. (Aricò et al., 2013) In that way the *RECK* expression is important because it upholds a constructive as well as a destructive balance. Both VEGF and MMPs are important for a variety of physiological processes, not least for tissue regeneration and wound healing but they are also the key to our understanding of tumor invasion. (Egeblad and Werb, 2002) 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 MMP are inhibited by *RECK* expression it implies that *RECK* plays a highly important and a much significant role in malignant cancer development.

There are still unsolved issues considering the role of RECK, especially the exact correlation between VEGF function and *RECK* expression. Also, even further in vivo animal studies are required for a better understanding of the multifunctional facets of the gene. Still, hitherto published studies suggest that RECK is a potential biomarker for early detection which may well be used for monitoring treatment in the future.

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