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# RECK—a newly discovered inhibitor of metastasis with prognostic significance in multiple forms of cancer

**Jonathan C. M. Clark · David M. Thomas · Peter F. M. Choong · Crispin R. Dass**

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**Abstract** The RECK (reversion-inducing cysteine rich protein with Kazal motifs) protein was initially discovered by its ability to induce reversion in ras-activated fibroblasts. The key action of RECK is to inhibit matrix metalloproteinases (MMPs) involved in breakdown of the extracellular matrix (ECM), and angiogenesis—namely MMP-2, MMP-9 and MTP-1. To this effect, it plays important physiological roles in embryogenesis and vasculogenesis. Additionally, it has a significant effect on tumorigenesis by limiting angiogenesis and invasion of tumours through the ECM. RECK has been studied in the context of a number of human tumours including colorectal, breast, pancreas, gastric, hepatocellular, prostate, and non-small cell lung carcinoma. In many of these tumours, RECK is down-regulated most likely as a result of inhibition at the Sp1 promoter site. MMP-2 and MMP-9 generally show an inverse association with RECK expression, but there are exceptions to this rule. Likewise, a reduction in tumour microvascular density (MVD) and VEGF have also been correlated with increased RECK levels, although more studies are required to define this effect. The predominant finding across all human tumour studies is a significantly improved prognosis (due to decreased invasion and metastasis) in tumours with preserved RECK expression. Although further research is required, RECK is a promising prognostic marker and potential therapeutic agent in multiple cancers.

**Keywords** Osteosarcoma · RECK · Metastasis · Angiogenesis · Sp1

J. C. M. Clark · P. F. M. Choong · C. R. Dass (✉)
Department of Orthopaedics, St. Vincent's Hospital,
University of Melbourne,
Melbourne, Australia
e-mail: crispin.dass@svhm.org.au

D. M. Thomas
Sarcoma Genomics and Genetics Laboratory,
Peter MacCallum Cancer Centre,
Melbourne, Australia

P. F. M. Choong
Bone and Soft Tissue Sarcoma Service,
Peter MacCallum Cancer Centre,
Melbourne, Australia

## 1 Introduction

RECK is a recently characterised membrane bound protein which, in the mouse, has been found to be important in suppressing two key components in the metastatic cascade: matrix metalloproteinases (MMPs), and angiogenesis [1]. A limited number of studies have also confirmed that RECK levels are significantly down-regulated in common human malignancies, compared with the normal surrounding tissue. By contrast, in the minority of tumour samples where RECK levels are normal or elevated, there is generally a reduction in local invasion, metastasis and an improved prognosis.

The protein RECK stands for 'reversion-inducing cysteine rich protein with Kazal motifs'. RECK was initially discovered by screening a human fibroblast cDNA library for genes giving rise to reversion-inducing clones when transfected into v-Ki-ras transformed NIH 3T3 cells [2]. Hybridization cDNA screening in the mouse identified a RECK gene producing a protein sequence of 93% homology.

The aim of this review is to provide an overall understanding of the mechanisms by which RECK inhibits tumour invasion, the molecular pathways involved in RECK

down-regulation, and further direction for RECK manipulation based on the patterns observed in some tumours.

## 2 Gene structure

The genomic structure for the RECK gene has been identified on chromosome region 9p13→p12 [3], and as noted by Kim et al. [4], the short arm of chromosome 9 contains a number of potential tumour suppressor genes [4], for example p16INK4a (CDKN2A) which has a well established role in melanoma. These authors demonstrated a loss of heterozygosity (LOH), an indicator of mutated tumour suppressor genes, in greater than 50% of small cell lung cancers at the 9p13 region, distinct from and centromeric to the CDKN2A locus. The identity of putative tumour suppressor gene(s) at the 9p13 locus remains unknown, but RECK represents an interesting candidate.

The gene sequence was identified by Eisenberg et al. [3] within two Bacterial artificial clones (BAC), using a BLAST search. It includes 21 exons and 20 introns, with 13 SNPs (single nucleotide polymorphisms). Four of these are found within the coding sequence which raises the possibility of disease involvement—where polymorphisms in the corresponding protein structure lead to abnormal function.

## 3 Protein structure

As originally described by Takahashi et al. [2], the protein coded for by the RECK gene is 971 amino acid residues in both humans and mice and there is 93% identity between the two species. The overall structure (Fig. 1) consists of a hydrophobic region at either end, a length of five cysteine repeats near the $NH_2$ end, two regions with epidermal growth factor (EGF)-like activity in the middle of the protein, and three regions with serine protease inhibitor activity. The COOH end allows glycosylphosphatidylinositol (GPI) anchoring to the cell membrane [2].

The first third of the protein sequence (marked by five cysteine repeats) appears to have particular functional importance in that it also contains a number of glycosylation sites at asparagine (Asn) residues. These sites are necessary for proper interaction with MMP-9 and MMP-2 as will be discussed later.

According to Takahashi et al. [2], the EGF-like regions have weak homology to the EGF protein sequence. EGF-like molecules are usually around 50–60 residues, they can bind to the EGF receptor and stimulate mitosis. EGF-like sequences within cell surface proteins, such as RECK, do not always bind to EGF receptors, but instead can influence cell development, adhesion, and protein interactions [5]. This functional description is certainly in line with the current understanding of RECK function and its interaction with MMPs.

Located within the middle of the protein structure are three serine-protease inhibitor-like domains (Fig. 1). SPIs (serpins) are proteins which inhibit the action of proteases by 'trapping reactions' and 'reversible tight binding interactions'. The first of these SPIs is identical to the Kazal motif, which is a particular family of peptidase inhibitors containing disulphide-rich proteins with small alpha and beta folds [6]. The other two SPIs in the RECK protein are similar but not identical to the Kazal motif. Given that MMPs are indeed proteases, these SPI-like domains are likely to have a significant role in MMP inhibition.

It appears that RECK is predominantly anchored to cell membranes *in vivo* via the COOH-terminal hydrophobic region and a GPI interaction. Serine proteases are known to be anchored to cell membrane via GPI and are thought to have some role in intracellular signal transductions [7]. This raises the possibility that RECK, being membrane-anchored

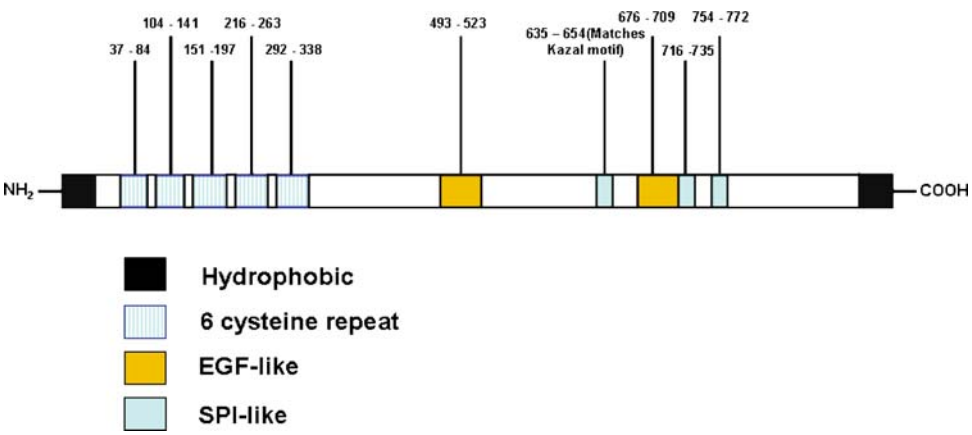


**Fig. 1** The overall structure of the RECK gene. Adapted from Takahashi et al. 1998 [2]

in the same way, may facilitate signal transduction also. RECK can be cleaved from the surface of cells *in vitro* using phosphatidylinositol-specific phospholipase C (PI-PLC) to make it available in soluble form [2].

Membrane anchoring of RECK is clearly of some importance, and the mechanisms behind this may be associated with its GPI interaction. Other proteins with similar GPI linkage to the membrane are thought to be involved in intracellular signal transduction. Noel and colleagues [7] mention this as a possible mechanism for serine proteases. Muller and coworkers [8] found that the phosphoinositol component of GPI-anchored proteins interacts with protein binding sites in glycolipid rafts and this results in signal transduction to produce an insulin-mimetic response.

## 4 Physiological role of RECK

RECK is an important mediator of tissue remodeling. Its main function is to inhibit MMP-2, MMP-9 and MT1-MMP post-transcriptionally [1]. These MMPs are active in breaking down the extra-cellular matrix (ECM) in both physiological and pathological states, including neoplasia [9]. RECK, on the other hand, keeps these processes under control. Researchers Oh et al. [1] found tissue disruption and reduced collagen IV, laminin and fibronectin in RECK −/− embryos when compared with tissues from the wild-type. This indicates that in the absence of RECK, MMPs degrade the matrix in an unchecked fashion.

RECK is required for normal embryonic development, facilitating ordered tissue remodeling by maintaining ECM around vessels and the neural tube [1]. Adequate ECM surrounding vessels promotes vessel stability and maturation. Mouse embryos lacking both copies of the RECK gene die slightly earlier than those lacking both RECK and MMP-2 genes because removing RECK alone leaves the proteolytic action of MMP-2 unchecked, resulting in excessive destruction of collagen IV and laminin in the basal lamina of vessel membranes [1]. RECK −/− embryos showed disrupted mesenchymal tissues and organogenesis. They had smaller bodies and developed abdominal haemorrhages. The vessels studied in these mice were primitive and underdeveloped, leaving the authors to conclude that RECK was more vital to stabilising angiogenic sprouting rather than later steps in maintaining vascular structure, which requires recruitment of cells to an area of vascular development.

## 5 Pathophysiological role of RECK

RECK over-expression in a fibrosarcoma cell line HT1080 compared with a control showed significant reduction in microvessel density (MVD) and branching [1]. The mechanism behind this, as mentioned above, is thought to be inhibition of MMP-2 which leads to reduced tissue remodeling and sprouting of vessels. Vessels in the RECK-expressing tumours tend to develop in diameter, but lack the ability to branch-off throughout the tumour tissue. This results in death of tumour tissue in areas of reduced MVD. The level of RECK expression did not appear to affect tumour volume, but mice with RECK-expressing tumours were noted to have a prolonged survival compared with the control.

The findings of reduced MVD correlate with results from the limited number of studies on human cancers where MVD has been examined in relation to RECK expression. There are three studies of note here, with somewhat conflicting results. Takeuchi et al. [10] identified an inverse relationship between RECK and both VEGF and MVD in colorectal cancer. Takenaka et al. [11] also found the same inverse relationship for MVD in non-small cell lung cancer but this relationship was only present in tissues strongly expressing VEGF, suggesting that the effects of RECK on vascular structures is directly or indirectly dependent on VEGF. A further study by Takenaka et al. [12] specifically looking at RECK expression in stage IIA N2 non-small cell lung cancer did not show significantly reduced MVD with high RECK expression. The reason for this is largely undetermined, but undoubtedly more studies will clarify the true extent of such an association.

In addition to its effects on angiogenesis, RECK also limits tumour invasion and metastasis by inhibiting matrix metalloproteinases (MMPs). MMPs were previously mentioned in relation to tissue remodeling where they facilitate ordered tissue break-down in concert with regulatory factors such as RECK. In pathological states such as neoplasia and rheumatoid arthritis, there is an imbalance in remodeling factors towards favouring of MMPs. The result is a destruction of the ECM allowing tumour cells to extend into surrounding tissues, and the bloodstream.

RECK appears to suppress MMPs secreted by tumour cells via a glycosylation mechanism. In normal cells, four separate asparagine residues in the RECK protein sequence are N-glycosylated. Simizu et al. [13] developed mutant RECK proteins where Asn residues were replaced with Glu in certain positions, to observe the effect of absent glycosylation on the expression of MMP-2 and MMP-9. They found that glycosylation of the $Asn^{297}$ was required to prevent MMP-9 secretion and $Asn^{352}$ glycosylation was needed to inhibit MMP-2 activation. This finding was then correlated with tumour cell invasion where HT1080-RECK mutants (absent glycosylation at $Asn^{297}$ and $Asn^{352}$) showed increased tumour cell invasion in comparison to HT1080 cells expressing wild-type RECK. $Asn^{297}$ is found within one of the cysteine knot regions of RECK (CKM5).

RECK sequences with mutated CKM5 were likewise found to be unable to suppress MMP-9.

## 6 Mechanisms by which RECK is down-regulated in cancer

The discovery of RECK and its ability to counteract MMPs has major implications for treating cancer. Importantly RECK inhibits both metastasis and angiogenesis, and it has been confirmed that in many tumours, RECK is down-regulated. The mechanism of this down-regulation appears to be multifactorial and also tumour specific, with a common target of inhibition being the Sp1 site on the RECK promoter sequence (Fig. 2—summary of RECK control). Early studies, Sasahara et al. [14, 15], speculated that by activating the extracellular signal regulated kinase (ERK) pathway, oncogenic Ras facilitates phosphorylation or other modification of Sp1/Sp3 factors which increases their affinity for the Sp1 site on the RECK promoter thus reducing RECK expression. Sasahara et al. [14] also hypothesised that histone deacetylation interaction with Sp1 may contribute to transcriptional repression of RECK. They used an inhibitor of HDAC, Trichostatin A (TSA), in NIH-3T3 cells but found no specific correlation with RECK levels. Transcription factor Sp1 has recently been shown to be overexpressed in a number of human cancers and its overexpression contributes to malignant transformation [16, 17]. Sp1 regulates the expression of a number of genes participating in multiple aspects of tumorigenesis such as angiogenesis, cell growth and resistance to apoptosis. Thus, the intricate interplay between Ras, Sp1 and RECK may dictate the degree of malignancy of a neoplastic cell.

Following on from the above study, Liu et al. [18] tested a similar hypothesis in CL-1 human lung cancer cells, and found increased cell surface RECK expression after adding TSA. This is thought to occur because TSA inhibits the interaction between HDAC and Sp1, and therefore reduces binding to the Sp1 promoter site. Additionally, once RECK was up-regulated, a corresponding inhibition of MMP-2 was noted.

A further study published by the same investigators, Chang et al. [19], demonstrates further evidence to support RECK transcription inhibition via ras and histone deacetylation. Interestingly, they found that Sp1 and Sp3 actually increase RECK promoter activity rather than inhibit it. Oncogenic ras activity, probably via the ERK phosphorylation pathway, resulted in increased Sp1 protein associated with HDAC, and this is believed to increase the binding of HDAC to the Sp1 site on the RECK promoter.

RECK is also inhibited by LMP-1, a product of the Epstein Barr virus (EBV) LMP-1 acts by binding to the Sp1 site in the promoter region of the RECK gene to inhibit promoter function. LMP-1 was also found to stimulate the ERK signaling pathway, but when this pathway was inhibited by PD98059 (ERK pathway inhibitor), there was diminished inhibition of RECK. This suggests that an overactive ERK signaling pathway (induced by LMP-1) may be responsible for RECK down-regulation [20].

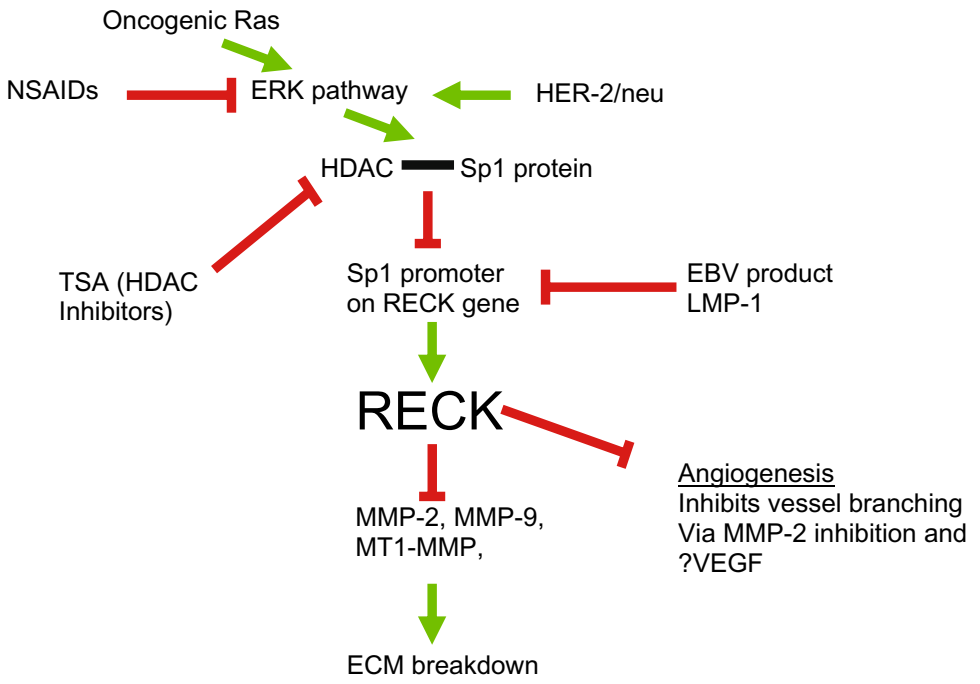


**Fig. 2** Summary of RECK control. RECK expression is inhibited via a number of different pathways acting on the Sp1 promoter site of the RECK gene. Specifically, oncogenic Ras increases ERK pathway activity leading to the combined action of histone deacetylase and the Sp1 protein in binding and inhibiting the Sp1 promoter site. NSAIDs and HDAC inhibitors act against this common pathway, and show potential for cancer therapy by reducing RECK down-regulation. The main function of RECK is to inhibit MMP-2, MMP-9, and MT1-MMP thereby reducing MMP destruction of the ECM and reducing metastasis. RECK also inhibits angiogenesis

In a very similar mechanism to that used by LMP-1, the HER-2/neu protein reduces RECK expression by increasing the binding of Sp1 proteins to the Sp1 site. It does this by inducing the ERK pathway to phosphorylate Sp1 proteins, increasing their affinity for the RECK promoter, and thus inhibiting RECK expression. Although this Sp1 protein action may not directly down-regulate RECK (as per Chang et al. [19]), HER-2/neu also recruits HDAC to Sp1 proteins and this combination represses expression of the RECK gene by binding to the promoter region [21].

Thus, it is evident from these three mechanisms that binding and inhibiting the Sp1 region of the RECK promoter provides the common pathway for down-regulating RECK expression in tumours. This process of inhibition can be achieved either through direct binding of the oncogenic factor (that is, LMP-1) or indirectly via up-regulation of the ERK signaling pathway which increases the affinity of proteins binding to the Sp1 region specifically HDAC.

There are almost certainly other pathways by which RECK is down-regulated in cancer. In their defining paper on RECK, Takahashi et al. [2] found RECK down-regulation in NIH3T3 cells transformed by a number of other oncogenes apart from ras [2]. These included v-fos, c-myc, v-src, v-fms, v-fes, and v-mos. Needless to say, closer attention to the molecular pathways intricately involved with RECK downregulation in cancer will no doubt lead to better ways of manipulating RECK expression via modulation of regulators upstream or reinstatement of molecular players downstream of RECK.

## 7 RECK down-regulation identified in many tumours

In studies over the last 6 years, a number of common tumours have been linked to RECK down-regulation, and in particular, down-regulation was associated with reduced survival. At least 19 human tumour cell lines have already demonstrated absent RECK mRNA [2]. Exceptions to this rule are being identified as RECK is analysed in a greater spectrum of tumours, for example in hepatocellular carcinoma, where RECK levels were actually higher than normal liver in 40% of specimens [22]. So far, tumours studied in relation to prognosis and RECK expression include; colorectal, breast, lung, gastric, hepatocellular and pancreatic carcinomas. The key findings from these studies will now be discussed briefly in turn (see Table 1 for summary).

In a study by Takeuchi et al. [10] colorectal carcinoma tissue samples obtained from 53 patients were analysed for RECK and MMP-9 using immunohistochemistry, with MMP-9 being found to be the predominant gelatinase on gelatin zymography. RECK was confirmed to be down-

**Table 1** Summary of RECK involvement in human cancer

| Parameters | Colorectal 1 | Colorectal 2 | Breast | Hepatocellular | Gastric | Lung—NSCL | Pancreatic (ductal adenocarcinoma) | Prostate | Osteosarcoma |
|---|---|---|---|---|---|---|---|---|---|
| High expression improves prognosis | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | NA |
| Down-regulated in specimens | Yes | Yes | Yes | No. 40.6% of tumours had high RECK expression | Yes | No. 44.9% strong 55.1% weak expression | No. 52% RECK positive, 48% RECK negative | Yes | Yes |
| Effect on angiogenesis | Decreased MVD and VEGF | NA | NA | NA | No correlation with VEGF | Significant | NA | NA | NA |
| Effect on level of MMPs | None | Decreased MMP-2. No effect on MMP-9 | NA | NA | Decreased MMP-9. No effect on MMP-2 and 7 | NA | Decreased MMP-2. No effect on MMP-9 | NA | Decreased pro MMP-2 activation |
| References | Takeuchi et al. [10] | van der Jagt et al. [23] | Span et al. [24] | Furumoto et al. [22] | Song et al. [28] | Takenaka et al. [11] | Masui et al. [27] | Riddick et al. [25] | Kang et al. [29] |

regulated in the majority of colon carcinoma specimens compared with surrounding normal tissues, and in tumour samples with high levels of expressed RECK there was no poorly differentiated tissue. Statistically significant findings included a higher frequency of lymph node metastases in the low-RECK group, and an inverse relationship between RECK levels and the Duke's staging. There was no relationship between RECK expression and MMP levels, however there was a correlation between prognosis and the RECK/MMP-9 ratio. This was based on the hypothesis that tumours with the highest RECK and the lowest MMP-9 levels, should display the least angiogenesis, invasion and metastasis.

A more recent study on RECK in colorectal carcinoma [23] found an inverse relationship between RECK and MMP-2 but not with MMP-9. This may be the result of preserved $Asn^{297}$glycosylation but absent glycosylation of $Asn^{352}$ (see section on "Pathophysiological role of RECK" above). These particular investigators believe that there is no direct inhibition of MMP-9 by RECK in colorectal cancer.

Span et al. [24] found significantly reduced RECK mRNA levels in invasive breast carcinoma specimens compared with normal surrounding tissues. Consistent with other studies, RECK expression had a significant bearing on survival times in these patients. Measurements of angiogenesis and MMPs were not performed in this study.

In a study by Riddick et al. [25], PCR was used to examine expression of MMPs and their inhibitors, which were either up or down-regulated in prostate cancer. Levels of these various factors were compared between benign and malignant prostate tissue. RECK and MMP2 were both significantly down-regulated in malignant tissue when compared with levels in the benign tumour tissue. The down-regulation of MMP-2 was an unusual finding, given that another study on prostate cancer shows increased MMP-2, although immunohistochemistry was used to study the MMP-2 protein rather than mRNA [26]. Riddick et al. [25] believe there are differences between the levels of observed MMP-2 mRNA and MMP-2 protein. This may relate to the fact that RECK is known to inhibit MMPs at the post-transcriptional level [2]. It is therefore more beneficial to study MMP protein rather than mRNA levels when looking at the effect of RECK. RECK down-regulation was associated with an increased Gleason score, which clearly demonstrates a correlation between low RECK and a worse prognosis. The main MMP up-regulated in malignant prostate cancer was MMP-26. So far, there appears to be no documented relationship between this MMP and RECK levels.

As previously noted, Furumoto et al. [22] found that in 26 cases of hepatocellular carcinoma, RECK expression was actually higher than in the non-tumour control specimens. Possible explanations for this will be addressed in the discussion. The direct correlation of RECK with improved prognosis was still evident in this study. With higher RECK levels identified in tumours, patients had better survival and tumours demonstrated reduced invasion.

When comparing NSCLC tumour groups with strong and weak expression of RECK there was no significant difference in the intra-tumour MVD [12]. In addition, no significant difference was found between strong and weak RECK expression in the categories of age, sex, tumour differentiation, clinical N factor, or number of N2 node stations involved. There was also no difference in proliferative index, apoptotic index or p53 expression. The most significant finding was that strong RECK expression correlated with improved survival overall (42.9% 5 year survival versus 23.1%), and in squamous cell carcinomas, weak RECK expression was associated with a poor prognosis. The level of angiogenesis was examined by Takenaka et al. [11], and it was found that MVD was reduced with high-expression of RECK but only in tumours concurrently expressing VEGF. This study covered NSCLC stages I–IIIA.

Similar to the pattern observed in hepatocellular carcinoma, RECK was not significantly down-regulated in pancreatic tumours studied by Masui et al. [27], with 52% actually demonstrating strong expression. An improved prognosis for RECK-expressing tumours was found in this study with patients whose tumours were positive for RECK having a 2 year survival of 42%, in comparison to 0% in the RECK-negative group ($P$=0.0463). Additionally, an inverse relationship was identified between RECK expression and MMP-2 activation ratio. On the other hand, RECK had no significant influence over MMP-9 in these pancreatic tumour samples, and as yet there is no explanation for this. Angiogenesis was not examined.

Similar to other cancers studied in relation to RECK, Song et al. [28] have identified an inverse relationship between RECK expression and gastric tumour invasion or metastasis. Increased RECK expression was associated with a reduction in MMP-9 levels, but not MMP-2 or MMP-7. This pattern is essentially the opposite from that seen in pancreatic cancer, where levels of MMP-9 were not affected by RECK.

Song et al. [28] also studied VEGF and found, not surprisingly, that levels of VEGF 121 and 165 were increased in most tumours, but these levels were not significantly reduced in the presence of high RECK expression. This finding was in contrast to colorectal and non-small cell lung cancer where VEGF levels and MVD were reduced with increased RECK. CD-34 immunohistochemistry was used to assess lymphatic invasion but MVD was not examined in this study, which may have shed more light on a potential anti-angiogenic action of RECK in gastric cancer.

Kang et al. [29] studied RECK in osteosarcoma cell lines which included the established lines HOS, U-2OS, MG-63,

and SaOS-2, and 23 lines established inhouse from patients prior to undergoing chemotherapy. They found that RECK mRNA levels were reduced in the majority of cell lines relative to expression in MG63 cells. Predictably, higher RECK levels were inversely correlated with pro-MMP-2, which was expressed across all cell lines. On the other hand, pro-MMP-9 was only expressed in U-2OS cells. Cell lines transfected with the RECK gene demonstrated that pro-MMP-2 activation was significantly decreased but pro-MMP-9 activation was not. Invasion assays were also conducted in matrigel (specifically using the HOS line), showing reduced cell invasion after RECK transfection.

Rheumatoid arthritis (RA) is a condition which exhibits similarities to invasive cancer in that MMPs play a significant role in ECM breakdown (particularly MT-MMP and MMP-14). In a study by van Lent et al. [30], RECK expression was significantly lower in RA synovial membranes compared with controls. RECK down-regulation in the synovium may be related to cytokine signaling proteins Sp1 and Sp3 which, as previously mentioned, are able to regulate RECK expression by binding to its promoter region. These researchers found no significant difference in MMP mRNA levels between rheumatoid tissues and controls. This does not rule out significant involvement of MMPs since the key regulatory point appears to be post-transcriptional [1].

## 8 Overall characteristics of RECK

When studying the relationship of RECK and MMPs in different tumours, there are a number of consistent observations. Firstly, RECK appears to have prognostic significance across the board. This finding alone may be used clinically to enable a more specific estimation of the tumour stage and grade at the initial biopsy. Secondly, RECK is noted to be down-regulated in the majority of tumours studied, with the exception of pancreatic and hepatocellular cancer. While more studies have to confirm this link, this currently provides a distinct avenue for further studies to reverse RECK knockdown, effectively over-expressing RECK in certain tumours and observing the effects on local invasion, metastasis, MVD, and angiogenic factors. Furthermore, while it may seem far-fetched at present, it may be possible to administer recombinant RECK (rRECK) to the patient either locally or systemically, or apply gene transfer techniques in order to improve prognosis.

There are still a number of features of RECK which need clarification. More studies are required to define the ability of RECK to inhibit angiogenesis and its interaction with VEGF in different human tumours. Only three studies so far, have examined this issue. The general pattern, consistent with Oh et al. [1], is a reduction in MVD—most likely through interaction with VEGF. However, as seen in gastric cancer [28], VEGF levels can be unchanged with RECK expression. These findings necessitate an exploration of the mechanisms behind RECK interaction with VEGF and the possible RECK-induced down-regulation of other angiogenic factors.

Various glycosylation sites within the RECK protein, and particularly the CKM5 region, are clearly important structural features relating to RECK's ability to inhibit MMP-9 and MMP-2. As we have seen, there are at least two tumours where RECK expression is not significantly reduced (hepatocellular and pancreatic carcinomas). In these cases, it may be that in some of the cells, RECK protein is actually mutated at the important glycosylation sites $Asn^{297}$ and $Asn^{352}$ rendering it unable to perform its usual role of inhibiting MMP-9 and MMP-2, but leaving it in large detectable quantities. More data is needed to determine the amount of specific MMP expressed in different human tumours, and to correlate this with the presence or absence of mutated forms of RECK. This will provide a clearer understanding of the mechanism of RECK's involvement in carcinogenesis.

The pre-dominant MMPs inhibited by RECK, MMP-2 and MMP-9, do not display a consistent inhibition across the different tumours studied. For example in colorectal cancer [10], there was no significant effect of RECK on the level of MMP-2 or MMP-9, even though tumours with the highest RECK/MMP-9 ratio were confirmed to have the best prognosis. In addition, the studies on gastric and pancreatic cancer show an opposing pattern of RECK interaction with MMP-2 and 9 (Table 1). Again, this may the result of glycosylation-site specific mutations in the RECK protein resulting in different MMP expression patterns.

## 9 RECK—potential for cancer therapy

While it is still early days. the consistent association of RECK with improved prognosis in multiple cancers suggests that this protein may have therapeutic potential. Such evaluation, initially in cell culture, then in preclinical (animal) models, and finally in clinical trials, will be necessary to demonstrate this. With this foresight, a number of therapeutic possibilities are now discussed.

Firstly, recombinant RECK could be used for therapy, similar to insulin and erythropoietin administration. This soluble RECK could then be administered either by systemic or by local infusion. There are several methods available for protein delivery, some even currently used clinically [31, 32]. Another possibility would be gene transfer, involving the delivery of viral or nonviral vectors

*in vivo* towards gene therapy. While this technique seems less likely given the limited control over the actual amount of RECK produced, studies with other proteins like PEDF demonstrate its latent promise [33]. With viral vectors though, there is the potential for further neoplastic change to occur when transfecting an already unstable genome [34]. Thus, use of non-viral vectors such as liposomes [35], microplexes [36, 37], cyclodextrins [38] and nanoparticles [39], may be more appropriate for gene transfer. What is needed now are studies outlining whether RECK is needed for normal development, apart from what is now known regarding its role in angiogenesis and vasculogenesis. The effects of RECK overexpression or treatment with rRECK on normal tissues should be elucidated in preclinical studies. Furthermore, its effects on developing versus established vasculature needs to be examined.

As previously outlined, the new evidence that HDAC inhibitors (that is, trichostatin A) are able to increase RECK levels by minimizing promoter inhibition, raises the possibility for HDAC inhibitors to be used therapeutically. Furthermore, there is the use of non-steroidal anti-inflammatories (NSAIDs) to increase RECK expression. Liu et al. [40] hypothesise that this observation may be due to NSAID inhibition of the ras/ERK/Sp1 pathway. They also note that the mechanism behind RECK up-regulation is independent of NSAID action on cyclooxygenase (COX) since concurrent administration of prostaglandin $E_2$ ($PGE_2$) or overexpression of COX-2 by transfection in the lung cancer cells, did not change the levels of RECK.

## 10 Conclusion

RECK offers significant promise as a future prognostic indicator and may be even as potential cancer therapeutic. By inhibiting MMP-2 and MMP-9, it inhibits the local tissue invasion and metastasis of a number of common human cancers, and also reduces angiogenesis. The ultimate test of its effectiveness is an improvement in prognosis, which so far, with the limited number of studies hitherto, has been demonstrated in nearly every human cancer evaluated.

RECK is down-regulated in many cancers, and where there are exceptions to this rule (that is, hepatocellular and pancreatic carcinoma), high RECK levels clearly offer a prognostic advantage. However, these cancers are not associated with a favourable prognosis overall [41, 42]. Although late diagnosis is probably the most significant factor in this contrasting observation, there may also be variable mutations of RECK glycosylation sites within tumour subclones such that one portion of cells cease to produce RECK (perhaps as a consequence of oncogenic ras), another produces effective unmutated RECK and still another portion produce ineffective mutated RECK at the $Asn^{297}$ and $Asn^{352}$ sites.

The molecular mechanisms behind RECK transcriptional regulation are clearly complex, and are still yet to be fully defined. However, with the discovery of the role of HDAC, via oncogenic ras, in reducing RECK transcription, the use of HDAC inhibitors such as TSA offers another possible means of manipulating RECK to provide cancer therapy. This may be a promising alternative to the difficulties faced by gene therapy. Additionally, new non-viral methods of transfecting cells may provide an effective method of up-regulating RECK production.

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