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Developmental Biology: Vasculogenesis is a Wreck Without RECK

Dispatch

Bryan Welm*, Joni Mott† and Zena Werb*

The unique membrane-associated inhibitor of matrix metalloproteinases, RECK, is required for vascular maturation during embryogenesis. The phenotype of a loss of function mutation of RECK shows the importance of pericellular proteolysis in development.

The complex network of proteins deposited around cells provides more than structural scaffolding: it contributes to numerous regulatory processes critical for development and pathology, including cellular organization, growth factor availability, angiogenesis and differentiation. Several protease families are involved in extracellular matrix (ECM) remodeling including the matrix metalloproteinases (MMPs) that contains 25 mouse and 22 human members [1]. MMPs can be divided into several groups based on their structure. Members of two of these groups contain structural motifs including transmembrane or glycophosphatidyl inositol-anchoring (GPI) domains that target them to the membrane or pericellular region. The developmental defect in mice lacking the MMP inhibitor, RECK, points to the importance of regulating pericellular MMP activity during development [2].

What makes RECK stand out from all other known MMP inhibitors is its membrane localization. RECK inhibits the activity of at least three MMP members including MMP-9, MMP-2 and MT1-MMP [2,3]. Interestingly, RECK also inhibits the release of proMMP-9 from the cell [3]. These data suggest that RECK can inhibit MMPs through several mechanisms including direct inhibition of protease activity, regulation of their release from the cell and possibly through sequestration of MMPs at the cell surface.

The activity of MMPs can be regulated by several distinct classes of proteins including the tissue inhibitors of metalloproteinases (TIMP1–4), the carboxy-terminal fragment of procollagen carboxy-terminal proteinase enhancer (CT-PCPE), α2-macroglobulin, tissue factor pathway inhibitor-2 (TFPI-2), and the NC1 domain of collagen IV [1,4–6]. These inhibitors are secreted and are localized to the ECM or found circulating in the plasma. Although the TIMPs share only 33–51% amino acid identity, their tertiary structure is highly similar due to the conserved positions of twelve cysteine residues that form six disulfide bonds. Correct pairing of the six cysteine residues in the amino terminus is critical for TIMP inhibition of MMP [7,8].

Figure 1. Alignment of TIMP with CT-PCPE, Netrin, secreted Frizzled related proteins and TFPI-2.

The human NTR modules [8] of the four TIMPs were aligned with the NTR modules of human CT-PCPE, human Netrin 2-like protein and secreted Frizzled-related proteins (human-secreted apoptosis-related protein, SARP-2 and SARP-3) and an internal region of TFPI-2. Sequence analysis was done using MultAlin [19] with a PAM250 scoring matrix in the web based resources of the Sequence Analysis and Consulting Service, a part of the Computer Graphics Laboratory at the University of California San Francisco. A consensus sequence is shown on the bottom line. High consensus amino acids are capitalized in red. Low consensus amino acids are lower case in blue. The symbol % is either F or Y.

The amino terminus of TIMP was recently predicted to be homologous to domains within other proteins having diverse functions (Figure 1). This protein motif, coined the NTR module, has a common feature of six conserved cysteines [9]. One predicted NTR module, CT-PCPE, inhibited MMP *in vitro*. Inhibition by CT-PCPE was hypothesized to occur because the folded structure of CT-PCPE was similar to TIMP [6]. Whether other NTR proteins such as Netrin and the secreted Frizzled-related proteins have MMP inhibitory activity remains to be determined. However it should be noted that chemical inhibitors of metalloproteinase activity synergize with Netrin as an axonal chemoattractant [10].

The mechanism by which TFPI-2 inhibits MMP is not clear. Although TFPI-2 does not seem to have a classic NTR module, there is a region within TFPI-2 containing a number of cysteine residues that aligns reasonably well with the amino-terminal domain of TIMP and NTR module polypeptides (Figure 1). RECK also contains a number of cysteine residues, however, comparison of the RECK amino acid sequence to TIMP and the NTR module does not show robust similarities. Thus, the biochemical mechanism for RECK inhibition of MMP remains to be elucidated

One of the unique features of RECK is that it contains a GPI domain making it a novel membrane-targeted MMP inhibitor. The targeting of several MMP

*Department of Anatomy, University of California, San Francisco, CA 94143-0452 USA. †Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA.

Correspondence: Bryan Welm, Department of Anatomy, BOX 0452, University of California, 513 Parnassus Avenue, San Francisco, CA 94143-0452, USA. email: bwelm@itsa.ucsf.edu

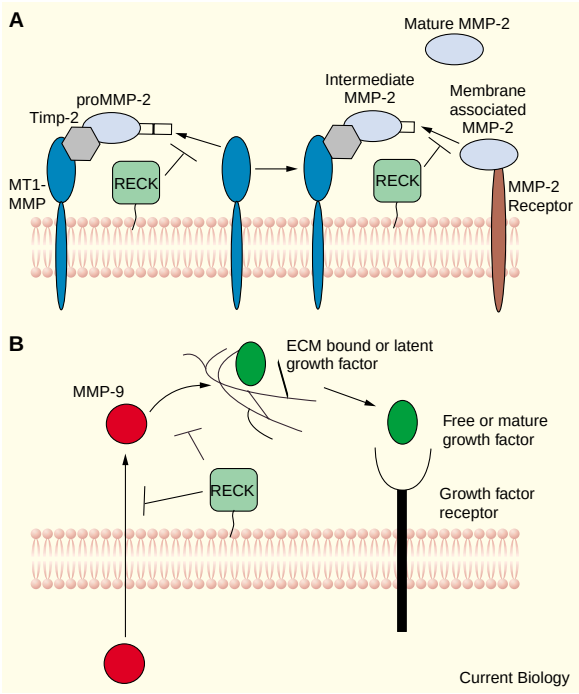

Figure 2. RECK can modulate the activity of MT1-MMP, MMP-2 and MMP-9 through several mechanisms.

(A) MT1-MMP is involved in the processing of proMMP-2 to produce mature MMP-2. During this process, TIMP-2 bridges MT1-MMP with proMMP-2. A second MT1-MMP molecule cleaves a portion of the prodomain on MMP-2 to form an intermediate MMP-2 protein. Further cleavage of MMP-2's prodomain by a different membrane associated MMP results in the final maturation and release of MMP-2 from the cell surface. Both of these processes can be inhibited by RECK. (B) RECK can also inhibit MMP-9's activity and its release from the cell through an unknown mechanism. Thus, RECK directly regulates MMP activity and can indirectly modulate localized growth factor availability.

family members to the pericellular region either by a direct membrane anchor, or by receptors or binding proteins at the cell surface, creates a region of concentrated proteolytic activity that may function in cell invasion as well as during localized growth factor signaling by modulating ECM–ligand interactions. Furthermore, the activation of some MMPs requires an interaction with certain membrane-associated MMPs. This paradigm has been nicely established for the activation of MMP-2 by the membrane associated MT1-MMP, a process which is regulated by both RECK and TIMP-2 (Figure 2).

Interestingly, TIMP-2 has two functions during MMP-2 activation, each confined to a distinct domain: as an MMP inhibitor through its amino-terminal domain which can bind to MT1-MMP; and as an MMP activator via its carboxy-terminal domain that recruits proMMP-2 to form a ternary complex which results in activation of MMP-2 (Figure 2). The effect of pericellular targeting positions RECK at the site of vigorous proteolytic activity that may potentiate its inhibitory ability (Figure 2). Furthermore, the GPI anchoring of RECK may target it to distinct cellular regions including caveolae or regions of cell adhesion, thus positioning it at critical sites involved in cell signaling. It is unknown whether RECK has other long-range functions as a consequence of its release from the cell surface by GPI cleaving enzymes such as glycosylphosphatidylinositol phospholipase C. These characteristics may, in part, explain some of the more profound developmental defects observed in *RECK* knockout mice when compared with the mild developmental phenotypes described for *TIMP-1*, *TIMP-2*, *TIMP-3* and *α2-macroglobulin* knockout mice [11–14].

Vascular development is a key process that must occur for embryonic development to proceed. The balance between ECM breakdown and deposition is critical for endothelial cell homeostasis and contributes to vasculogenesis and angiogenesis. During vasculogenesis, periendothelial cells proliferate and migrate to form networks of immature vessels. Mural cells (precursor pericytes) stimulate vascular maturation by stabilizing nascent vessels through the deposition of ECM [15]. The ECM can have multiple effects on endothelial cells including inhibiting proliferation and migration suggesting that modulating MMP processing and proteolytic activity is critical for vascular maturation. Indeed, during development RECK is expressed in large blood vessels and mural cells [2] similar to the pericyte marker smooth muscle α-actin. Thus expression of RECK in pericytes positions it to elicit potent and concentrated inhibitory effects on MMP-9, MT1-MMP and MMP-2 activity near endothelial cells.

In the absence of RECK, increased proteolytic activity would be expected to result in ECM degradation and the destabilization of nascent vessels. This effect is seen in *reck*$^{-/-}$ embryos that display an embryonic lethality at about 10.5 days and a defect in vascular maturation [2]. Moreover, it is noteworthy that embryonic survival can be extended by one day, a significant delay at this critical stage of vascular development, when *reck*$^{-/-}$ mice are bred into the *MMP-2*$^{-/-}$ background suggesting that abrogating MMP-2 activity partially rescues the phenotype [2]. The question remains as to whether activity of the other two MMPs — MMP-9 and MT1-MMP — can account for the remaining phenotype, or whether RECK has other enzymatic targets such as other MMPs, or members of the metalloproteinase superfamily such as ADAMs, ADAM-TSs or Tolloid-related Astascins [2].

One function of the ECM — once thought to be its only function — is to provide a physical barrier between tissue compartments. ECM is also an important reservoir of growth, differentiation and angiogenic factors, and cleavage of ECM can produce fragments that have distinct biological activities [16]. During invasive developmental growth, for example during angiogenesis, restructuring of the ECM is required for blood vessel migration and intrusion into neighboring tissue. ECM degradation also occurs during pathological events including tumor growth and invasion, and ECM degrading proteases such as MMPs are involved in these processes. Upregulated MMP expression is seen in almost all human cancers and their expression generally correlates with poor prognosis and with malignant transformation [17]. The converse correlation

with cancer would be predicted for the expression of TIMPs. However, this is not necessarily the case as elevated TIMP expression is observed in several human cancers and its detection often correlates with poor prognosis [17]. Indeed, conflicting data exist on TIMP expression and tumorigenesis in both mouse models and human cancers [1]. TIMPs may have multiple roles, including activating and inhibiting MMP activity as well as MMP-independent influences, depending on which TIMPs are expressed and their expression levels.

While the role of TIMPs in tumorigenesis may be somewhat confusing, MMP activity, particularly pericellular activity, appears vital for tumor angiogenesis. When RECK is expressed in human fibrosarcoma cells and these cells are transplanted into nude mice, tumors arise that have angiogenic defects [2]. These data suggest that the loss of RECK expression in tumor cells enhances angiogenesis. However, although RECK is attached to the membrane its sphere of influence appears widespread; it is capable of mediating effects between tumor cells and the vascular endothelium. This broad effect is most likely a result of RECK's multifaceted ability to regulate MMP-9 secretion, and inhibit MT1-MMP and MMP-2 activity at the cell surface (see Figure 2). As MT1-MMP is the major activator of MMP-2, RECK's inhibitory ability is, in essence, amplified. From gain-of function and loss-of-function experiments, Oh *et al.* [2] predict a model in which RECK is required for vascular development, yet too much RECK could block sprouting angiogenesis by inhibiting endothelial cell invasion through surrounding ECM.

Is RECK a tumor suppressor? *RECK* was originally identified as a gene that can revert the transforming phenotype of *ras* transformed NIH3T3 cells [3]. Interestingly, *RECK* mRNA expression in culture is inhibited by *ra*s as well as by several other oncogenes commonly found altered in tumors including *myc, mos, fos* and *src* [3]. Furthermore, *RECK* expression is low or absent in several tumor cell lines, and *RECK* reduces invasiveness and metastatic potential when transfected into metastatic fibrosarcoma and melanoma cells lines [3]. In human hepatocellular carcinoma samples, *RECK* expression correlates with less invasive tumors as well as better prognostic outcome [18]. Further genetic analysis of *RECK* in human tumors is needed to determine if this gene is truly a tumor suppressor, but with the limited data we already have, it is intriguing to speculate that an MMP inhibitor may one day obtain this classification.

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