Collapsin response mediator protein-1: A novel invasion-suppressor gene

Jin-Yuan Shih¹, Yuan-Chii G. Lee², Shuenn-Chen Yang³, Tse-Ming Hong³, Chi-Ying F. Huang² & Pan-Chyr Yang¹−³

¹Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; ²National Health Research Institutes, Taipei, Taiwan; ³Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan

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Abstract

Numerous genetic changes are associated with metastasis of cancer cells. Previously, we used microarray to identify that collapsin response mediator protein-1 (CRMP-1) was involved in cancer invasion and metastasis. We further characterized that CRMP-1 was a novel invasion-suppression gene. Members of the CRMP gene family are intracellular phosphoproteins involved in the mediation of semaphorin induced F-actin depolymerization and growth cone collapse. The precise mechanism by which CRMP-1 inhibits invasion is not yet clear. However, CRMP-1 transfected cells had fewer filopodia and less Matrigel-invasion abilities. A low expression of CRMP-1 mRNA in lung cancer tissue was significantly associated with advanced disease, lymph node metastasis, early post-operative relapse, and shorter survival. In this article, we reviewed the functions of CRMPs and semaphorins and analyzed the structure and motifs of CRMP-1 by bioinformatics. As such, we hoped to shed further light on the mechanism by which CRMP-1 suppresses the invasion of cancer cells.

Abbreviations: CRMP – collapsin response mediator protein; cDNA – complementary DNA; DHPase – dihydropyrimidinase; DRG – dorsal root ganglion; GluNAc – β-N-acetylglucosamine; mRNA – messenger RNA; NGF – nerve growth factor; NLS – nuclear localization signal; PLD2 – phospholipase D2; sema – semaphorin.

Introduction

Tumor dissemination is a complex process. The pathogenesis of cancer metastasis consists of a series of linked, sequential, and selective steps. Invasion, which is the most crucial step in the metastatic cascade, contains a series of biological activities involving the interaction of tumor cells with the surrounding environment [1, 2]. Tumors are a mass of heterogeneous neoplastic cells with different properties [3, 4]. During cancer progression, some tumor cells acquire new characters, either as an expression of metastasis-promoting mutations or due to the loss of metastasis-suppressing genes. With all tumor progression, there is a movement towards a more aggressive behavioral pattern.

Invasion suppressor gene: Collapsin response mediator protein-1

Identification by cDNA microarray

We were interested in searching for novel genes associated with the invasion or metastasis of lung cancer. We previ-
cDNA into a highly invasive cell line, CL_{1-5}, and assessed the changes of invasion phenotypes. CL_{1-5} originally had an elongated morphology, whereas CRMP-1 transfected CL_{1-5} cells had a rounded morphology, similar to CL_{1-0}. CRMP-1 transfected CL_{1-5} cells also had fewer filopodia, closer to the number on CL_{1-0} cells [11]. The differentially expressed CRMP-1 gene was confirmed further by the quantitative real-time reverse-transcription polymerase chain reaction of messenger RNA (mRNA) from 80 lung cancer patients who underwent surgical resection to evaluate the association between the expression of CRMP-1 and clinical results. The expression of CRMP-1 mRNA in non-small-cell lung cancer was significantly lower statistically than in adjacent normal tissue. When the median value of CRMP-1 mRNA was used to classify patients into high-expression or low-expression groups, low-expression patients were found to have a more advanced disease state and lymph node metastases. On the other hand, the high-expression group had a significantly longer disease-free and overall survival period than the low-expression group [11].

**CRMPs as intracellular mediators of semaphorin/collapsin signaling**

Members of the CRMP family of phosphoproteins may mediate semaphorin/collapsin-induced growth cone collapse and are involved in both axonal guidance and neuronal differentiation (Figure 1). CRMP family members have a 50%–70% amino acid sequence homology. During the last few years, five members of the CRMP gene family (CRMP-1, CRMP-2, CRMP-3, CRMP-4, and CRMP-5), encoding closely related 60–66 kDa proteins, have been independently cloned by several different laboratories pursuing different goals [12–19]. This implies that CRMPs may have various functions and each CRMP may have a unique function. The nomenclature, however, was a little perplexing because each laboratory cloned and named them independently. The members of this family have been referred to as CRMP (collapsin response mediator protein), TOAD-64 (turned on after division 64 kDa protein), Ulip (UNC-33 like phosphoprotein), DRP (dihydropyrimidinase related protein) and TUC (TOAD/Ulip/CRMP). Nonetheless, the most frequently used name in medical literature is CRMP.

CRMPs are expressed mainly in the nervous system, especially during embryogenesis [16]. Immunocytochemical studies have shown that CRMPs are distributed in the lamellipodia and filopodia of the growth cone, the shaft of axons, and the neuronal cell body [15, 18, 19]. Their expression and phosphorylation are spatially and temporally regulated during development although their molecular mechanisms of action are yet to be clearly. Accumulated evidence shows that CRMP is a critical molecule that induces the growth cone collapse of neuronal cells.

CRMP bears a sequence homology to UNC-33 [14], a nematode protein, whose absence produces aberrant elongation of axons and uncoordinated movement in the worm *Caenorhabditis elegans* [20]. Chick CRMP-62 (CRMP-2) was identified by its involvement in the semaphorin 3A/collapsin-1-induced mediation of growth cone collapse in chick dorsal root ganglion neurons [15]. CRMP-2, the most widely studied member, is reported to mediate through a signal transduction cascade involving heterotrimeric G proteins [15]. Recently, it was reported that CRMP-2 is also involved in the lysophosphatidic acid-induced growth cone collapse of dorsal root ganglion neurons via Rho-associated protein kinase [21].

**Semaphorin-neuropilin signals**

Semaphorins are a large family of secreted and transmembrane signaling proteins that regulate axonal guidance. Recent studies suggest that semaphorins also act in such diverse processes as signaling the immune system, control of cell motility, lung branching morphogenesis, and tumor progression [22–25]. Neuropilins and plexins act as receptors for semaphorins [26].

More than 20 different semaphorins have been identified and these have been classified into eight groups. The best-characterized class is the class 3 semaphorins. Currently, six members have been identified (Sema3A–F). Sema3A, also known as collapsin-1, was the first member to be identified and was the prototypic member of this family. Sema3A acts as a diffusible, repulsive guidance cue *in vivo* for neurons [27]. Sema3A may also participate in neural crest cell migrations at the earlier stages of development [28]. It inhibits growth cone motility and the migration or motility of endothelial cells by depolymerization of F-actin and retraction of lamellipodia [29, 30]. Filopodia are the first motors to pull the leading edges of the cell forward [31].

Neuropilin-1 and plexin A1 are required for Sema-3A to initiate the signal transduction cascade. This intracellular cascade seems to involve the monomeric G protein, Rho family GTPase, CRMP family, LIM kinase and F-actin depolymerization [15, 21, 32]. Some direct connections between CRMPs and Rho protein signaling have been elucidated. However, the mechanism by which the semaphorin signal is transduced to the cytoskeletal machinery is not well studied yet.

Neuropilin-1 is a membrane protein with three distinct functions: (1) mediator of cell adhesion via a heterophilic molecular interaction; (2) receptor of class 3 semaphorin that mediates the semaphorin-elicted inhibitory signals into the neuron; (3) receptor of VEGF_{165} that regulates vessels formation [33]. Sema3A inhibits the binding of VEGF_{165} to neuropilin-1 and vice versa. VEGF_{165} and Sema3A are competitive inhibitors that have overlapping neuropilin-1 binding sites [30, 34]. Neuropilin also acts as co-receptor, it can bind to KDR/flk-1 to form the active receptor for VEGF_{165} and facilitates angiogenesis. This interaction has been reported to mediate vascular branching morphogenesis in development and endothelial cell chemotaxis and mitogenesis, the latter being a potential promoter of metastasis. Neuropilin-1 overexpression in the rat prostate cancer model results in larger tumors associated with substantially increased tumor angiogenesis and a lesser degree of tumor cell apoptosis [35]. Induction of neuropilin-1 expression in tumor cells enhances tumor cell motility as well as VEGF_{165} binding capacity, both of which increase the possibility of tu-