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The Structure of BRMS1 Nuclear Export Signal and SNX6 Interacting Region Reveals a Hexamer Formed by Antiparallel Coiled Coils

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We present here the first structural report derived from breast cancer metastasis suppressor 1 (BRMS1), a member of the metastasis suppressor protein group, which, during recent years, have drawn much attention since they suppress metastasis without affecting the growth of the primary tumor. The relevance of the predicted N-terminal coiled coil on the molecular recognition of some of the BRMS1 partners, on its cellular localization and on the role of BRMS1 biological functions such as transcriptional repression prompted us to characterize its three-dimensional structure by X-ray crystallography.

The structure of BRMS1 N-terminal region reveals that residues 51–98 form an antiparallel coiled-coil motif and, also, that it has the capability of homo-oligomerizing in a hexameric conformation by forming a trimer of coiled-coil dimers. We have also performed hydrodynamic experiments that strongly supported the prevalence in solution of this quaternary structure for $BRMS1_{51–98}$.

This work explores the structural features of BRMS1 N-terminal region to help clarify the role of this area in the context of the full-length protein. Our crystallographic and biophysical results suggest that the biological function of BRMS1 may be affected by its ability to promote molecular clustering through its N-terminal coiled-coil region.

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Abbreviations used: BRMS1, breast cancer metastasis suppressor 1; SNX6, sorting nexin 6; NES, nuclear export signal; NLS, nuclear localization signal; DOSY-NMR, diffusion-ordered spectroscopy nuclear magnetic resonance; DLS, dynamic light scattering; PDB, Protein Data Bank.

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Introduction

Most human cancer deaths are caused by metastasis, a process involving several defined steps including detachment of cancer cells from a primary tumor, invasion of surrounding tissue, survival in the bloodstream, extravasation and colonization at distant organs.

Many genetic events are required to promote metastasis including several genes that show relative reduced expression levels in aggressive human tumors. Restoration of normal levels for some of these proteins in a metastatic tumor cell line results in a significant reduction of the metastatic behavior *in vivo* with no effect on primary tumor growth.[1] Little is known about the molecular events triggered by this family of genes known as metastasis suppressors. Nevertheless, its function seems to become increasingly relevant toward the final stages of the metastatic cascade.[2,3]

Breast cancer metastasis suppressor 1 (BRMS1), a member of this growing metastasis suppressor family, was identified by differential display and significantly reduced metastasis without affecting breast,[4] melanoma[5,6] or ovarian carcinoma[7] primary tumor growth.

BRMS1 transcriptional repression reduces metastasis and appears to be epigenetically regulated.[8,9] Molecular mechanisms of action for BRMS1 include restoration of cell–cell communication[10] and abrogation of phosphoinositide signalling.[11] It has been proposed that BRMS1 might induce all these changes by playing a role in transcription.[12–19] In fact, BRMS1 has the ability to form complexes with several proteins involved in the regulation of transcription.[18,20,21] Thus, BRMS1 can interact with a wide range of proteins in order to promote different effects on the metastatic cascade. Despite the wide biomedical interest, molecular characterization of BRMS1, including structural studies, remains largely elusive. Analysis of the BRMS1 primary structure predicts a mostly disordered protein showing a few putative motifs, including two nuclear localization signals (NLSs) in two predicted coiled-coil regions (Fig. 3d).

Coiled-coil regions have long been known as protein–protein interaction modules that can exert biologically relevant functions.[34,35] In fact, BRMS1 second predicted coiled-coil domain, including residues 130–187, is involved in interactions with AT-rich interactive domain 4A, part of the SIN3: histone deacetylase chromatin remodeling complex.[18] In addition, we have recently shown that BRMS1 N-terminal putative coiled coil interacts with a predicted coiled-coil region of sorting nexin 6 (SNX6) including residues 300–406, increasing BRMS1-dependent transcriptional repression.[21] Moreover, we have also shown that BRMS1 residues 74–91 have been identified as a functional nuclear export signal (NES).[33] Given that this predicted N-terminal coiled coil has an important role on the molecular recognition of some of the BRMS1 partners,[18,21] that it contains a sequence essential for its cytoplasmic localization,[33] and that it has also been related to important BRMS1 biological functions such as transcriptional repression, we wanted to characterize its three-dimensional structure. We had previously reported the crystallization of BRMS1 including residues 51–84[36] and now have solved the structure of the predicted BRMS1 coiled-coil region including residues 51–98 ($BRMS1_{51–98}$).

Structural characterization of this BRMS1 fragment reveals a trimer of antiparallel binary coiled coils. The crystal structure is in agreement with the solution oligomerization state determined by analytical ultracentrifugation and hydrodynamic experiments. This particular molecular arrangement might suggest the ability of BRMS1 to promote molecular clustering through its N-terminal coiled-coil region.

Results

$BRMS1_{51–98}$ crystal structure

Human BRMS1 N-terminal coiled-coil region (residues 51–98) was successfully expressed in *Escherichia coli* Rosetta(DE3)pLysS cells and purified by metal-chelating affinity chromatography followed by size-exclusion chromatography in a buffer containing 20 mM Tris–HCl (pH 7.5) and 50 mM NaCl. The elution volume of $BRMS1_{51–98}$ in the pre-calibrated gel-filtration column corresponded to an apparent molecular mass of ~32 kDa (data not shown). A major peak of protein, followed by absorption at 280 nm, was obtained at an elution volume of 160 mL. After analysis of more than 900 crystallization conditions and hit optimization, typical crystals grew with dimensions of 0.05 mm × 0.05 mm × 0.05 mm. Further improvement based mainly on the screening of different pH, precipitant concentration, temperature growing and protein:reservoir ratio volume led to 0.25 mm × 0.25 mm × 0.25 mm crystals after 3 days. Prior to data collection, crystals (Fig. 1a) were vitrified in the presence of paratone.

Diffraction data were collected and reduced to 1.9 Å. The data statistics of the X-ray diffraction experiments are shown in Table 1. Crystals are rhombohedral and belong to the *R*32 space group with unit cell dimensions, in the hexagonal setting, of $a = 60.4$ Å, $b = 60.4$ Å and $c = 133.5$ Å and $\alpha = 90.0°$, $\beta = 90.0°$ and $\gamma = 120.0°$.

The asymmetric unit contains two $BRMS1_{51–98}$ molecules, chains A and B, arranged as a left-