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ACCA Phosphopeptide Recognition by the BRCT Repeats of BRCA1

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The tumour suppressor gene *BRCA1* encodes a 220 kDa protein that participates in multiple cellular processes. The BRCA1 protein contains a tandem of two BRCT repeats at its carboxy-terminal region. The majority of disease-associated *BRCA1* mutations affect this region and provide to the BRCT repeats a central role in the BRCA1 tumour suppressor function. The BRCT repeats have been shown to mediate phospho-dependant protein-protein interactions. They recognize phosphorylated peptides using a recognition groove that spans both BRCT repeats. We previously identified an interaction between the tandem of BRCA1 BRCT repeats and ACCA, which was disrupted by germ line *BRCA1* mutations that affect the BRCT repeats. We recently showed that BRCA1 modulates ACCA activity through its phospho-dependent binding to ACCA. To delineate the region of ACCA that is crucial for the regulation of its activity by BRCA1, we searched for potential phosphorylation sites in the ACCA sequence that might be recognized by the BRCA1 BRCT repeats. Using sequence analysis and structure modelling, we proposed the Ser1263 residue as the most favourable candidate among six residues, for recognition by the BRCA1 BRCT repeats. Using experimental approaches, such as GST pull-down assay with Bosc cells, we clearly showed that phosphorylation of only Ser1263 was essential for the interaction of ACCA with the BRCT repeats. We finally demonstrated by immunoprecipitation of ACCA in cells, that the whole BRCA1 protein interacts with ACCA when phosphorylated on Ser1263.

Keywords: BRCT; ACCA; phosphopeptide; protein modelling; protein–protein interaction

Introduction

BRCA1 acts as a tumour suppressor gene, and germ line mutations in this gene are found in a large proportion of families with breast and ovarian cancers.[1] It encodes a 220 kDa protein that participates in a number of cellular processes such as cell cycle checkpoint control, DNA damage repair, transcriptional regulation, and protein ubiquitination.[2] BRCA1 contains a tandem of two BRCT domains (BRCT repeats) at its carboxy-terminal region.[3–5] The majority of *BRCA1* mutations associated with an increase susceptibility to breast and ovarian cancer affect the BRCT repeats, resulting in truncated products lacking one or two BRCT repeats.[6] These findings, together with the observation that deletion of the Brca1 BRCT repeats is responsible for tumour development in mice,[7] demonstrate that BRCT repeats play a central role in the BRCA1 tumour suppressor function. Several proteins interact with this region and might collaborate functionally with BRCA1. For example, BRCA1 binds RNA polymerase holoenzyme, CtIP and histone deacetylases, all implicated in transcription regulation.[8–12] More recently, the BRCT repeats of BRCA1 have been shown to interact with the DNA helicase BACH1, implicated in response to

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Abbreviations used: BRCA1, breast cancer susceptibility gene 1; BRCT, BRCA1 C terminus; ACCA, acetyl CoenzymeA carboxylase alpha; FAS, fatty acid synthase; AMPK, AMP-activated protein kinase; GST, glutathione-*S*-transferase; wt, wild-type.

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DNA damage,[13] and with acetyl-CoA carboxylase alpha (ACCA), the rate-limiting enzyme for long chain fatty acid synthesis.[14]

The BRCT repeats of BRCA1 have been shown to recognize phosphorylated peptides.[15–18] The phospho-dependent binding specificity of peptides extends from the pSer/pThr(+1) to the pSer/pThr(+5) position with a particular strong selection for aromatic residues (Phe) in the pSer/pThr(+3) position. Several structural studies have revealed the mechanism by which the recognition of the phosphorylated peptide by BRCA1 occurs, involving a groove spanning the BRCT repeats.[19–23] A pocket within the amino-terminal BRCT repeat accommodates the phosphorylated serine, whereas a largely hydrophobic groove located at the interface between the two BRCT repeats recognizes the phenylalanine. The phosphopeptide of BACH1,[20–22] of CtIP[23] and an optimized phosphopeptide,[19] bind the BRCA1 binding groove in an extended conformation.

We recently identified ACCA as a novel binding partner of BRCA1.[14] Notably, this interaction is mediated by the BRCT repeats of BRCA1 and is disrupted by germ line *BRCA1* mutations that affect these repeats. We further showed that BRCA1 affects ACCA activity through interacting *via* its BRCT repeats in a phospho-dependent manner.[24] However, the precise phosphorylated form of ACCA that binds the BRCT repeats of BRCA1 remained to be delineated. Here, we searched for potential phosphorylation sites in the ACCA sequence which might be recognized by the BRCA1 BRCT repeats.

Results

The search for potential phosphorylation sites in ACCA which might be recognized by the BRCA1 BRCT repeats was based on the known sequence and structural features of phosphopeptides recognized by BRCA1, which are described above. On the sequence level, we found that six serine residues of ACCA have a phenylalanine in the (+3) position (Table 1). The secondary structures of the phosphopeptides are various, as deduced from structural data for domains that could be accurately modelled on known experimental structures (biotin carboxylase and carboxyltransferase domains) or predicted from the sequence, using PSI-PRED[25] and hydrophobic cluster analysis[26] (Table 1).

Worth noting is the strict identity of the four "core" amino acid residues between the BACH1 peptide and the ACCA peptide containing Ser1263, making it the most favourable candidate for recognition by the BRCA1 BRCT repeats on the sequence level. Remarkably, the second core of four amino acids identified today in phospho-binding partners of BRCA1, namely that of the CtIP peptide containing Ser327 (SPVFGATS),[27] resembles that of BACH1 and shares with the ACCA 1263 peptide an alanine in position p(Ser)/p(Thr)+5. The predicted exposed nature of the ACCA peptide in a coil conformation would make it particularly amenable, at the secondary and tertiary structure level, to interact with BRCA1 in a way similar to the BACH1 peptide. This hypothesis was supported by the three-dimensional model of the ACCA 1263 peptide in complex with the BRCA1 BRCT repeats, based on the available three-dimensional structure of the BACH1–BRCA1 complex (Figure 1).

As noticed above, models of the three-dimensional structure of the domains in which ACCA peptides are included can be obtained for five peptides out of six (peptides 344, 432 (biotin carboxylase domain) and 1585, 1952 and 2211 (carboxyltransferase domain)), based on the experimental structures of the yeast enzymes (see Materials and Methods) (Figure 2). The only peptide for which this information is not accessible is the peptide containing Ser1263, which was discussed above. These models show that, on a static point of view (thus without considering the possibility of conformational mobility), the peptides generally make part of regular secondary structures, thus restricting their ability to be free to interact in an extended conformation with the BRCT repeats. This is the case for peptides including serine residues 344 and 432 (beta strands) and residues 1585 and 2211 (alpha helices). Moreover, the phenylalanine is generally buried within the core of the structures (peptides 344, 432, 1952 and 2211).

We also investigated the accessibility of the considered five peptides within the whole protein domain structures to the BRCA1 BRCT repeats, by superimposing them to the BACH1 peptide bound to the BRCA1 BRCT repeats (data not shown).

Table 1. Features of the ACCA candidate peptides

ACCA serine	Related domain	Sequence (BACH1: **SPTF**NKQT)	Secondary structure (solvent accessibility of Phe)[a,b]	BRCT domains accessibility[a,b]
344	Biotin carboxylase	**SP**I**F**VMRL	Beta-strand (buried)[a]	−
432	Biotin carboxylase	**S**FY**F**LELN	Beta-strand (buried)[a]	−
1263	–	**SPTF**EAGH	Coil (exposed)[b]	+
1585	Carboxyltransferase	**S**KR**F**QAQS	Alpha helix (exposed)[a]	±
1952	Carboxyltransferase	**S**GF**F**DYGS	Coil (buried)[a]	−
2211	Carboxyltransferase	**S**RT**F**FYWR	Alpha helix (buried)[a]	−

Identities relative to the BACH1 peptide are indicated in bold and underlined.
[a] Modelled on the basis of the experimental structures of the corresponding domains in the yeast enzyme.
[b] Predicted from sequence.