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Review

Phosphopeptide interactions with BRCA1 BRCT domains: More than just a motif

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ABSTRACT

BRCA1 BRCT domains function as phosphoprotein-binding modules for recognition of the phosphorylated protein-sequence motif pSXXF. While the motif interaction interface provides strong anchor points for binding, protein regions outside the motif have recently been found to be important for binding affinity. In this review, we compare the available structural data for BRCA1 BRCT domains in complex with phosphopeptides in order to gain a more complete understanding of the interaction between phosphopeptides and BRCA1-BRCT domains.

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1. Introduction: from BRCT domain to the BRCA1 tandem BRCT domains

Maintaining the integrity of genetic information is key to the survival of cells. Eukaryotes have evolved to host sophisticated cell-cycle-dependent regulation networks to deal with DNA damage arising from both exogenous and endogenous sources. Dynamic protein–protein interactions and protein complex assemblies in the signalling cascades mediating DNA damage response (DDR) are often initiated by protein post-translational modification such as phosphorylation. Reversible interaction interfaces are created by phosphorylation on serine/threonine residues by key DDR-signalling-cascade regulators that include phosphoinositide 3-kinase-related kinases (PIKKs) (ATM, ATR, DNA-PK) (Matsuoka et al., 2007; Meek et al., 2008) and checkpoint-effector kinases (Chk1, Chk2 and MK2) (Reinhardt and Yaffe, 2009).

Deficiency in DDR regulation networks caused by gene mutation could potentially lead to cell death or tumorigenesis. Mutations in the tumour suppressor gene *BRCA1* (breast cancer susceptibility gene 1) were first identified in patients with hereditary breast and ovarian cancer (HBOC) (Futreal et al., 1994; Hall et al., 1990; Miki et al., 1994). Further analysis of this 1863-amino-acid BRCA1 protein led to the identification of the BRCT (named as **BR**CA1-**C**-

Abbreviations: DDR, DNA damage response; PIKKs, phosphoinositide 3-kinase-related kinases; *BRCA1*, breast cancer susceptibility gene 1; HBOC, hereditary breast and ovarian cancer; BRCT, BRCA1-C-Terminal; PPII, polyproline II.

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Terminal) domain (Koonin et al., 1996). Even though BRCT domains share low sequence identities (about 14%), they have been successfully identified in many proteins involved in RNA processing, cell checkpoint regulation and DNA damage response and repair (Bork et al., 1997; Callebaut and Mornon, 1997; Woods et al., 2012), where BRCT domains mediate interactions with proteins, DNA (Yamane and Tsuruo, 1999; Yamane et al., 2000) and poly(ADP-ribose) (PAR) (Pleschke et al., 2000).

A typical BRCT domain comprises between 90 and 100 amino-acid residues and folds as a globular domain with secondary structural elements ordered as βαββαβα. The first crystal structure of a BRCT domain, that of XRCC1, defined a four-stranded parallel β-sheet surrounded by three α-helices, in which helices α1 and α3 locate on one side of the β-sheet and α2 on the other side (PDB: 1CDZ) (Zhang et al., 1998) (Fig. 1a). 23 human genes have been identified encoding BRCT domain-containing proteins and 12 of these contain more than one BRCT domain within the sequence (Mesquita et al., 2010; Woods et al., 2012), as does BRCA1 where the two BRCT domains are packed tightly in tandem. One of the most significant properties for BRCA1 BRCT domains is their ability to bind phosphorylated proteins containing the sequence motif pSXXF (where p indicates phosphorylation) (Manke et al., 2003; Rodriguez et al., 2003; Yu et al., 2003) (Fig. 1b). Potential phospho-independent interaction has also been reported recently for interaction of BRCA1-BRCT domains with DNA-PKcs (Davis et al., 2014). Together with the N-terminal RING domain, C-terminal BRCT domains are the sites of the main BRCA1 mutations found in patients with breast and ovarian cancer (Couch and Weber, 1996; Friedman et al., 1994; Shattuck-Eidens et al., 1995). Studies of mouse models have shown that BRCT-domain phosphoprotein binding but not the RING-domain E3-ligase activity is required for Brca1 tumour suppression (Shakya et al., 2011). Comprehensive reviews of the evolution and function of BRCT domains can be found in Leung and Glover (2011), Mesquita et al. (2010). Here, we focus on the interface between the phosphopeptide and BRCA1 BRCT domains.

2. 2D-structure comparisons of BRCA1 tandem BRCT domains bound with phosphopeptides

The availability of protein structure data for BRCA1 tandem BRCT domains alone and in complex with various phosphopeptides has enabled us to understand and compare the protein-peptide interaction in detail. We first describe these interactions using a “2D interaction map” method, built upon the structural interaction fingerprint methodology used in the CREDO structural interactomics database (Schreyer and Blundell, 2013) (Fig. 2). All bound phosphopeptides identified in protein structures were sequence aligned, with the phosphorylated Ser residue, which mediates key H-bonds in the pSXXF motif, placed at position 0. The side chain of the Phe at the +3 position inserts into a deep hydrophobic binding pocket, forms hydrogen-bond interactions via its mainchain atoms, and interacts with the sulphur in BRCT M1775 via a ring π interaction. Outside the pSXXF motif region, the N- and C-terminal peptides also contribute to the interaction. The structure of Bach1 in complex with a phosphopeptide (PDB: 1T29) (Shiozaki et al., 2004) defines an especially large interface outside the motif. The optimized phosphopeptide in the BRCA1 BRCT-phosphopeptide complex (PDB:1T2V) (Williams et al., 2004) has a Tyr at position −3 in the N-terminal region, which forms hydrophobic and ring-atom interactions through its side chain. The “2D interaction map” in Fig. 2 provides a clear visualization for comparing phosphopeptide interactions with BRCT domains as well as for defining the depth of the binding pockets.

3. The “two anchors” binding in pSXXF motif region

The 3D structures of BRCA1-tandem-BRCT domains show that the two BRCT domains (BRCT1 and BRCT2) are associated in a head-to-tail manner (Williams et al., 2001). A large hydrophobic interface between BRCT1 and BRCT2 is created by α2 (from BRCT1) and α′1 and α′3 (from BRCT2), with an extra linking helix αL between the two domains, next to the α′3 of BRCT2. Using the BRCA1-Bach1 structure (PDB: 1T29) (Shiozaki et al., 2004) as an example, Bach1 phosphopeptide can be seen to bind to the cleft generated between two tightly packed BRCT domains (Fig. 1b). The pSXXF motif in Bach1 phosphopeptide functions as a “two-anchor” interaction mode (also described as “two-knobs” in Shiozaki et al. (2004))) with the BRCT domain, in which the phosphorylated Ser (position 0) and the Phe (position +3) interact with the BRCT1 and BRCT2 individually (Fig. 2b). Comparison with a BRCT-domains-only structure (PDB: 1JNX) (Williams et al., 2001) by structural superposition and alignment with the BRCT1-domain structure shows that the BRCT2 domain moves closer to BRCT1 when bound to a phosphopeptide (Fig. 2b). A larger degree of phosphopeptide-induced domain movement was also observed in TopBP1 BRCT 7/8- Bach1 complex (Leung et al., 2010). This suggests that

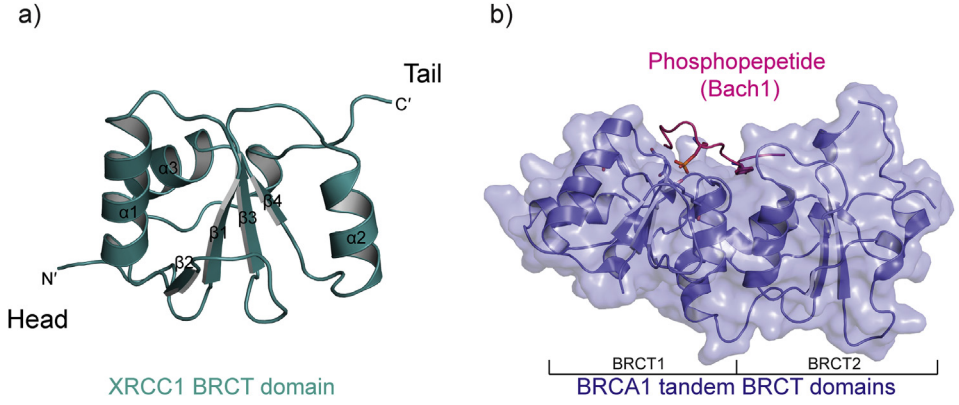

Fig. 1. BRCT domain structures. a) Crystal structure of the second BRCT domain of XRCC1 (PDB: 1CDZ) (Zhang et al., 1998). The ribbon representation of the mainchain is in dark green. N′ (Head) and C′ (Tail) ends are labelled. b) Crystal structure of BRCA1-BRCT tandem 1 and 2 domains in complex with Bach1 phosphopeptide (PDB: 1T29) (Shiozaki et al., 2004). Bach1 phosphopeptide binds to the cleft between the two BRCT domains. The ribbon representation of BRCT domain mainchain is in slate colour and the globular structure is represented by a transparent surface. Bach1 phosphopeptide is in pink.