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CCDC98 is a BRCA1-BRCT domain–binding protein involved in the DNA damage response

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The product of the breast cancer-1 gene, *BRCA1*, plays a crucial part in the DNA damage response through its interactions with many proteins, including BACH1, CtIP and RAP80. Here we identify a coiled-coil domain–containing protein, CCDC98, as a BRCA1-interacting protein. CCDC98 colocalizes with BRCA1 and is required for the formation of BRCA1 foci in response to ionizing radiation. Moreover, like BRCA1, CCDC98 has a role in radiation sensitivity and damage-induced G2/M checkpoint control. Together, these results suggest that CCDC98 is a mediator of BRCA1 function involved in the mammalian DNA damage response.

To survive and maintain their genomic integrity, cells are equipped with the ability to sense and respond to DNA damage[1,2]. The importance of this surveillance system has been demonstrated by the finding that inactivation of the DNA damage response can lead to cancer-susceptibility syndromes and neoplastic transformation. Many proteins, including the protein kinase ataxia-telangiectasia mutated (ATM), phosphorylated histone H2AX (γH2AX) and mediator of DNA damage checkpoint-1 (MDC1), are involved in sensing, transducing and responding to DNA damage signals[3]. The product of *BRCA1* is also a checkpoint mediator, and its BRCT domains function in this process by interacting with phosphoserine or phosphothreonine motifs[4–6]. Previous studies have shown that the BRCA1-BRCT domains are important for BRCA1's functions in tumor suppression[7] and the DNA damage response[8–10]. In the presence of DNA lesions, BRCA1 participates in many DNA damage response pathways, including cell-cycle checkpoints during S phase and at the G2/M transition, and DNA repair via homologous recombination[8–11]. Defects in these checkpoints and DNA repair may underlie the increased tumorigenesis observed in patients with *BRCA1* mutations.

Although BRCA1 is known to be recruited to DNA breaks and to participate in checkpoint regulation, it is not yet clear how the recruitment of BRCA1 is controlled in the cell. To gain further insights into the regulation of BRCA1 upon DNA damage, we sought to identify previously unknown BRCA1-BRCT domain–binding proteins using a tandem affinity-purification approach. Here we report that human CCDC98 protein associates with BRCA1 and demonstrate that CCDC98 acts upstream of BRCA1 and regulates the BRCA1-dependent DNA damage signaling pathway.

RESULTS

CCDC98 is a BRCA1-associated protein

To identify additional BRCA1-associated proteins, we purified BRCA1-BRCT domain–containing complexes from human embryonic kidney 293T cells stably expressing a BRCA1-BRCT domain with an N-terminal triple tag comprising an S tag, a Flag epitope and a streptavidin-binding peptide (SFB–BRCA1-BRCT). Mass spectrometry revealed a number of known BRCA1-associated proteins, including BRCA1-associated C-terminal helicase (BACH1), CtBP-interacting protein (CtIP) and receptor associated protein-80 (RAP80)[6,12–15]. In the same experiment, we also identified several putative BRCA1-associated proteins (**Supplementary Table 1** online). Among these, we paid special attention to a coiled-coil domain–containing protein, CCDC98. This protein contains an SPTF motif at its extreme C terminus; an identical sequence in BACH1 is required for interaction of BACH1 with BRCA1-BRCT domains[6]. The physiological function of CCDC98 is unknown. Notably, we also identified CCDC98 as a RAP80-associated protein in a tandem affinity purification of RAP80-containing complexes (**Supplementary Table 2** online), confirming that CCDC98 and RAP80 interact. As both CCDC98 and RAP80 exist in BRCA1-containing complexes (**Supplementary Tables 1** and **2**), we speculated that these three proteins might form a complex.

BRCA1 specifically binds the SPTF motif of CCDC98

We confirmed the association of CCDC98 with BRCA1 and RAP80 using coimmunoprecipitation experiments (**Fig. 1a**). In addition, bacterially expressed glutathione *S*-transferase (GST)-tagged BRCA1-BRCT domain and GST-RAP80 pulled down CCDC98 from cell extracts (**Fig. 1b**), again confirming that CCDC98 interacts with both BRCA1 and RAP80. Notably, although CCDC98 interacted with the BRCA1-BRCT domain in a phosphorylation-dependent manner, its association with RAP80 was phosphorylation independent (**Fig. 1b**).

Prompted by this phosphorylation-dependent interaction between BRCA1-BRCT and CCDC98, we examined whether the C-terminal SPTF motif of CCDC98 is required for its interaction with BRCA1-BRCT. GST–BRCA1-BRCT specifically bound wild-type CCDC98 and

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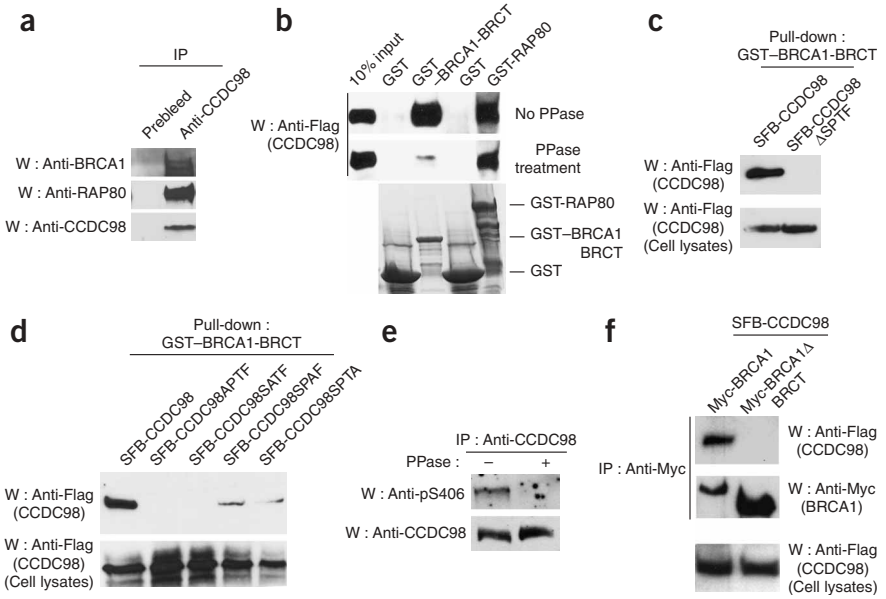

Figure 1 Identification of CCDC98 as a BRCA1-binding protein. (**a**) The interaction between endogenous CCDC98 and BRCA1 or RAP80. Immunoprecipitation (IP) reactions were done using preimmune serum (prebleed) or anti-CCDC98. Western blotting analyses (W) were done with indicated antibodies. (**b**) Phosphorylation-dependent interaction between BRCA1-BRCT and CCDC98. GST, GST–BRCA1-BRCT or GST-RAP80 was incubated with cell lysates containing exogenously expressed Flag-tagged wild-type CCDC98, with or without phosphatase. Bound CCDC98 was analyzed by anti-Flag immunoblotting. Lower gel shows amounts of proteins used in these experiments. (**c**,**d**) Beads with GST–BRCA1-BRCT were incubated with cell lysates containing exogenously expressed SFB-tagged wild-type CCDC98, CCDC98ΔSPTF or SPTF point mutants with the C-terminal sequences indicated in their names (CCDC98APTF, CCDC98SATF, CCDC98SPAF and CCDC98SPTA). Bound CCDC98 proteins were analyzed by anti-Flag immunoblotting. (**e**) CCDC98 is phosphorylated at Ser406. IP reactions using anti-CCDC98 were followed by mock or phosphatase treatment. Western blotting was done with indicated antibodies. (**f**) 293T cells were transfected with plasmid encoding Myc-BRCA1 or Myc-BRCA1ΔBRCT and with plasmid encoding SFB-CCDC98. Cell lysates were subjected to immunoprecipitation and immunoblotting with indicated antibodies (upper blots). Lower blot shows amounts of SFB-tagged CCDC98 in lysates.

did not bind CCDC98 lacking the C-terminal SPTF sequence (CCDC 98ΔSPTF; **Fig. 1c**). We also generated several point mutations in the SPTF motif of CCDC98. Whereas GST–BRCA1-BRCT specifically pulled down wild-type CCDC98, its affinities for the CCDC98 point mutants were greatly diminished (**Fig. 1d**). Using a phosphospecific antibody against the Ser406 residue in the SPTF motif, we confirmed that this serine residue is indeed phosphorylated *in vivo* (**Fig. 1e**). This phosphorylation and the BRCA1-CCDC98 interaction did not change after DNA damage (data not shown). Only wild-type BRCA1, and not a BRCA1 variant lacking the BRCT regions (BRCA1ΔBRCT), associated with CCDC98 *in vivo* (**Fig. 1f**). Together, these data suggest that CCDC98 binds BRCA1 in a phosphorylation-dependent manner through an interaction between BRCA1-BRCT and the C-terminal SPTF motif of CCDC98.

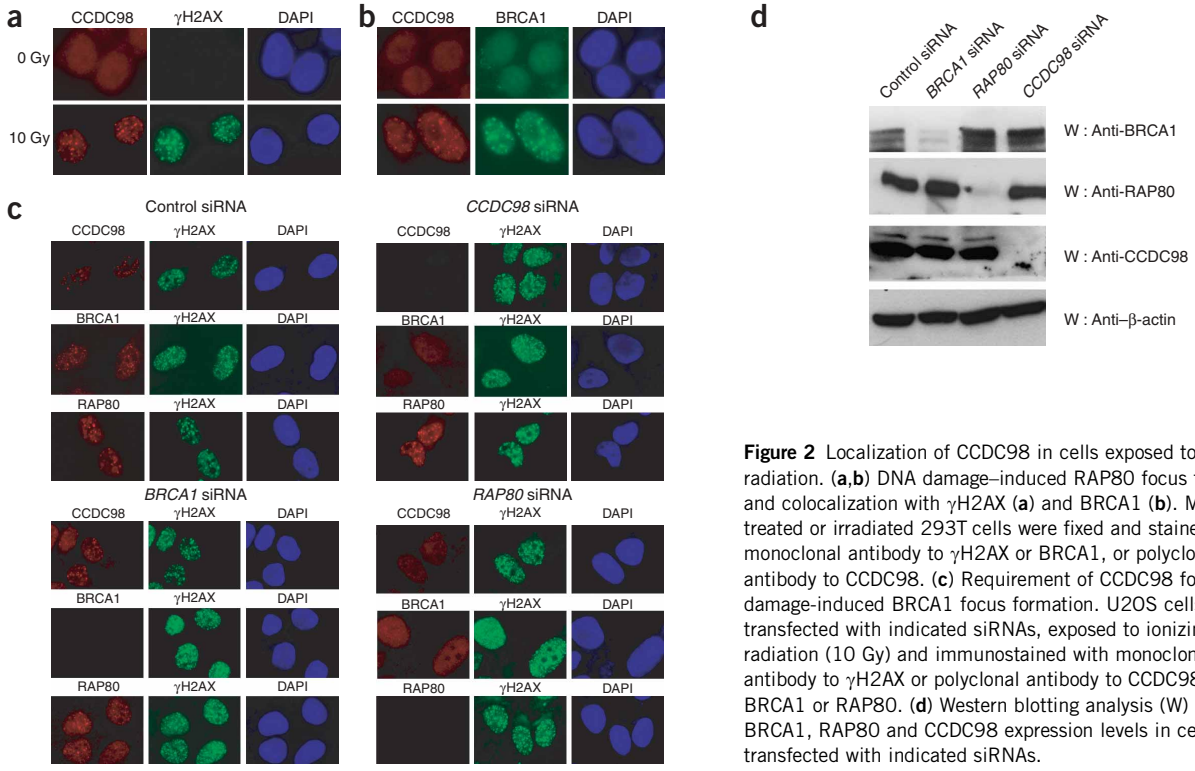

Figure 2 Localization of CCDC98 in cells exposed to ionizing radiation. (**a**,**b**) DNA damage–induced RAP80 focus formation and colocalization with γH2AX (**a**) and BRCA1 (**b**). Mock-treated or irradiated 293T cells were fixed and stained with monoclonal antibody to γH2AX or BRCA1, or polyclonal antibody to CCDC98. (**c**) Requirement of CCDC98 for damage-induced BRCA1 focus formation. U2OS cells were transfected with indicated siRNAs, exposed to ionizing radiation (10 Gy) and immunostained with monoclonal antibody to γH2AX or polyclonal antibody to CCDC98, BRCA1 or RAP80. (**d**) Western blotting analysis (W) of BRCA1, RAP80 and CCDC98 expression levels in cells transfected with indicated siRNAs.

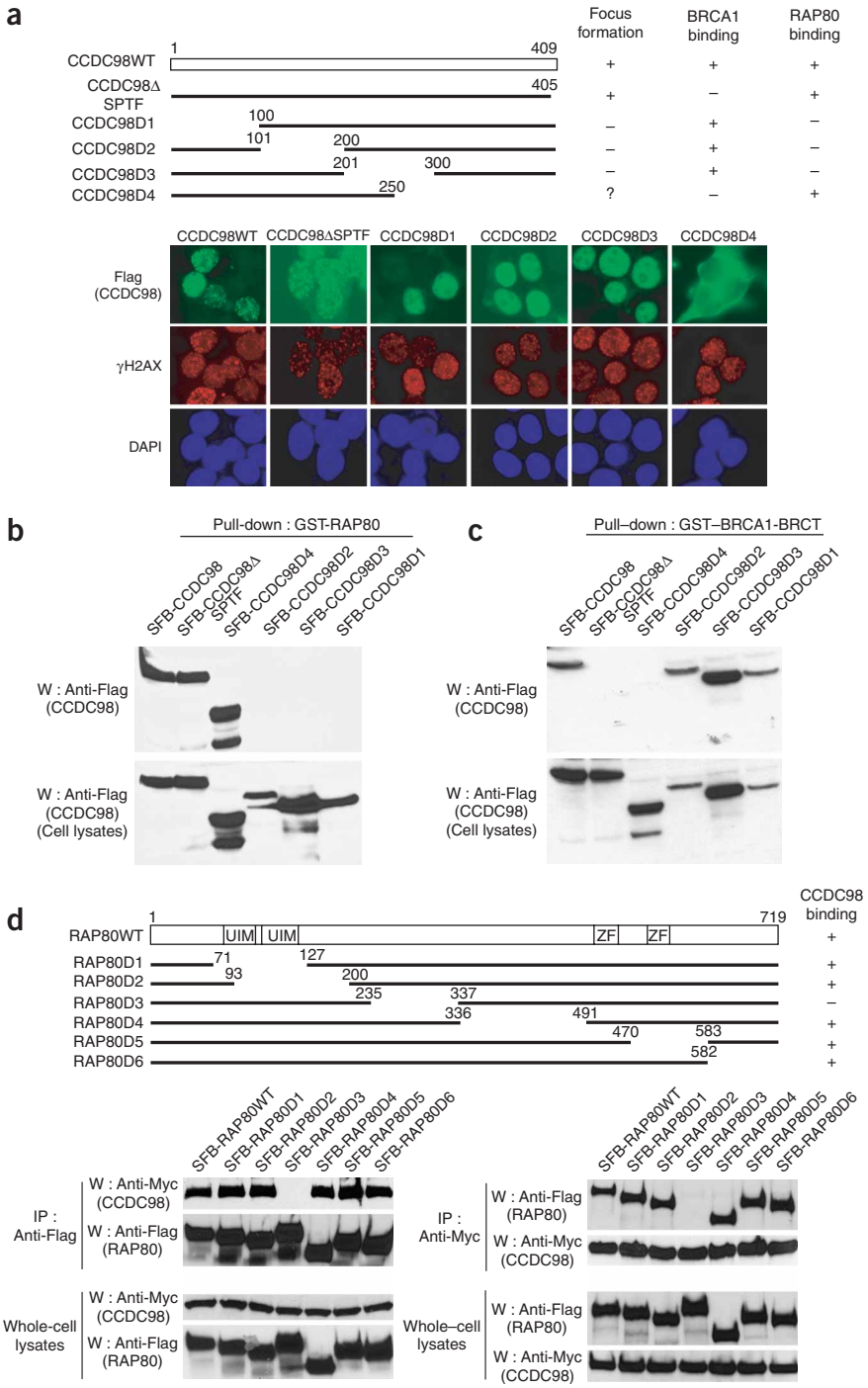

Figure 3 Focus localization of CCDC98 depends on its N-terminal RAP80-binding region. (**a**) 293T cells were transfected with SFB-tagged wild-type (WT) CCDC98 or deletion mutants shown in diagram. After 24 h, cells were exposed to 10 Gy of ionizing radiation. Eight hours after irradiation, cells were fixed and stained with monoclonal anti-Flag or polyclonal anti-γH2AX. (**b**,**c**) Mapping of the RAP80- and BRCA1-interacting domains in CCDC98. Beads coated with GST-RAP80 (**b**) or GST–BRCA1-BRCT (**c**) were incubated with cell lysates containing exogenously expressed SFB-tagged WT CCDC98 or deletion mutants. After extensive washing, bound RAP80 was analyzed by western blotting (W) with anti-Flag. (**d**) SFB-tagged WT RAP80 and its internal deletion mutants were used to map the CCDC98-interacting domain in RAP80. 293T cells were transfected with plasmids encoding Myc-CCDC98 and the indicated SFB-RAP80 proteins. Cell lysates were subjected to immunoprecipitation (IP) and immunoblotting with indicated antibodies (top blots). Bottom blots show amounts of SFB-RAP80 and Myc-CCDC98 in these lysates.

CCDC98 and BRCA1 form foci after DNA damage

As BRCA1 localizes to sites of DNA breaks in cells exposed to ionizing radiation, we checked the localization of CCDC98 before and after DNA damage. Using an antibody to CCDC98, we found the protein to be evenly distributed in the nucleoplasm of normal cells (**Fig. 2a**). After cells were exposed to ionizing radiation, CCDC98 localized to DNA damage–induced foci and colocalized with γH2AX (a marker of DNA damage) and BRCA1 (**Fig. 2a**,**b**). This indicates that the localization of CCDC98, like that of BRCA1, is regulated in response to DNA damage. Notably, we discovered that BRCA1 did not accumulate at DNA breaks in cells where *CCDC98* messenger RNA was depleted using short interfering RNA (siRNA); however, the localization of RAP80 to damage sites was normal in these cells (**Fig. 2c**). Moreover, formation of both BRCA1 and CCDC98 foci was abolished in *RAP80*-depleted cells, but formation of CCDC98 and RAP80 foci was normal in *BRCA1*-depleted cells (**Fig. 2c**). As a control, we showed that the expression level of BRCA1 is the same with or without *CCDC98* knockdown (**Fig. 2d**). In addition, *RAP80* knockdown also does not change the expression of CCDC98 or BRCA1 (**Fig. 2d**). Collectively, these data suggest that CCDC98 acts downstream of RAP80 but upstream of BRCA1 in the DNA damage response pathway.

CCDC98 focus formation depends on its N terminus

Our results suggested that CCDC98 forms a complex with RAP80 and BRCA1 and localizes to sites of damaged DNA. Next, we attempted to determine which regions of CCDC98 are important for its localization to foci. Full-length CCDC98 and CCDC98ΔSPTF mutant localized normally to nuclear foci in cells with DNA damage, whereas all of the other CCDC98 deletion mutants we tested did not (**Fig. 3a**). All three N-terminal and internal deletion mutants of CCDC98 also did not bind RAP80 (**Fig. 3b**), whereas CCDC98ΔSPTF and a CCDC98 mutant with a large C-terminal deletion (CCDC98D4) were defective in BRCA1 binding (**Fig. 3c**). Because it localizes to the cytoplasm, it is difficult to interpret the results obtained with the CCDC98D4 mutant (two putative nuclear localization sequences, 358-Lys-Arg-Ser-Arg-361 and 368-Lys-Arg-Ser-Lys-371, are deleted in this mutant). Nevertheless, these data suggest that CCDC98 mediates the interaction between BRCA1 and RAP80

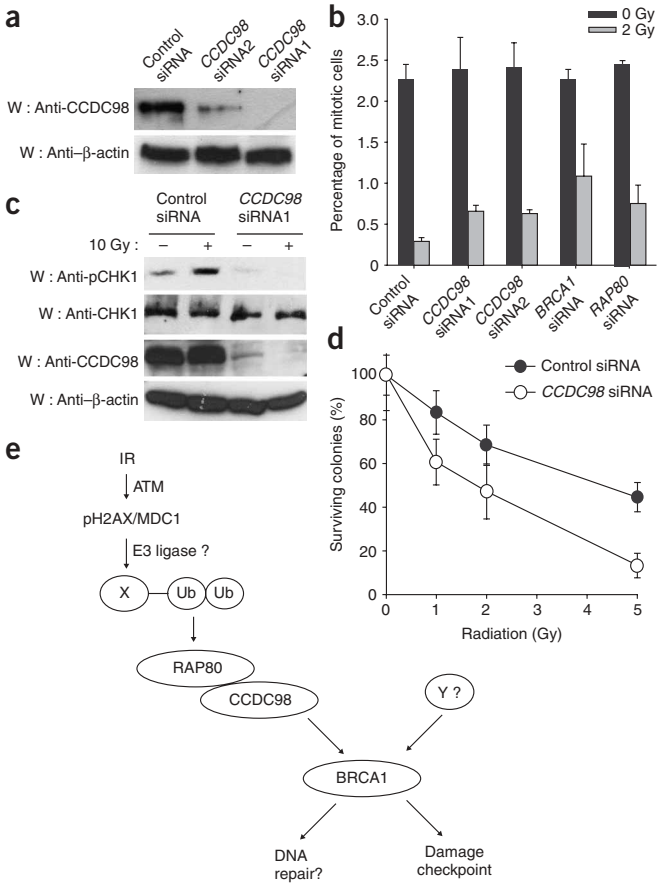

Figure 4 Requirement of CCDC98 for ionizing radiation–induced DNA damage response. (**a**) Western blotting analysis (W) of CCDC98 expression in cells transfected with indicated siRNAs. (**b**) G2/M checkpoint control in *CCDC98* knockdown cells. HeLa cells transfected with indicated siRNAs were exposed to 0 or 2 Gy of ionizing radiation. Cells were fixed and stained with histone-specific anti-pH3 (a mitotic marker) and propidium iodide. Percentages of mitotic cells were determined by FACS analysis. Data shown are averages of three independent experiments; error bars indicate s.d. (**c**) Requirement of CCDC98 for CHK1 phosphorylation after DNA damage. Control or *CCDC98* siRNA–transfected HeLa cells were exposed to 0 or 10 Gy of ionizing radiation, harvested 2 h later and immunoblotted with indicated antibodies. (**d**) Radiation sensitivity of cells lacking CCDC98. HeLa cells were transfected with control or *CCDC98* siRNAs. Cells were irradiated with indicated doses of ionizing radiation. Percentage of colonies surviving was determined 10–12 d later. Experiments were done in triplicate; results shown are averages of two or three independent experiments at each dose; error bars indicate s.d. (**e**) Model of the DNA damage response pathway that integrates CCDC98. Our data indicate that CCDC98 operates upstream of BRCA1 and specifically regulates BRCA1 localization and function after DNA damage.

and that the localization of CCDC98 to foci depends on its interaction with RAP80.

We confirmed a strong interaction between CCDC98 and RAP80 using a baculovirus-insect cell system (**Supplementary Fig. 1** online). Using a series of deletion mutants of RAP80, we identified a region (residues 235–337) on the C-terminal side of the ubiquitin-interacting motifs (UIMs) that is required for its interaction with CCDC98 (**Fig. 3d**). The same region of RAP80 is also important for its association with BRCA1 *in vivo* (**Supplementary Fig. 2** online), an observation which agrees with our proposal that CCDC98 bridges the interaction between RAP80 and BRCA1.

CCDC98 is required in the G2/M checkpoint

The loss of BRCA1 leads to defects in the DNA damage response—in particular, impaired cell-cycle checkpoints and increased sensitivity to DNA damaging agents[16,17]. We therefore examined whether the loss of CCDC98 results in similar defects in the DNA damage response. Both *CCDC98* siRNAs we synthesized efficiently decreased CCDC98 expression in cells (**Fig. 4a**). Cells treated with these siRNAs showed defective G2/M checkpoint control after DNA damage (**Fig. 4b** and **Supplementary Fig. 3** online). The protein kinase CHK1 acts downstream of BRCA1 and is required for this G2/M checkpoint control in response to ionizing radiation[18–21]. If CCDC98 functions upstream of BRCA1, a defect in CHK1 activation is expected in cells depleted of CCDC98. This is indeed the case (**Fig. 4c**). *CCDC98* knockdown cells were also more sensitive to radiation than cells transfected with control siRNA (**Fig. 4d**). These data indicate that CCDC98 is a key upstream regulator that influences BRCA1 function upon DNA damage (**Fig. 4e**).

DISCUSSION

In this study, we identified CCDC98 as a BRCA1-BRCT–binding protein. Like BRCA1, CCDC98 normally exists in the nucleoplasm but localizes to DNA breaks after exposure to ionizing radiation. CCDC98 also participates in the BRCA1-dependent G2/M checkpoint control, suggesting that CCDC98 functions together with BRCA1 in the DNA damage response.

Besides CCDC98, the ubiquitin-interacting protein RAP80 was also identified as a BRCA1-associated protein in our biochemical purification of BRCA1-containing complexes. Studies from several groups, including ours, have demonstrated that RAP80 acts upstream of BRCA1 and regulates BRCA1 localization and function after DNA damage[13–15]. Moreover, another group has also identified CCDC98 (called Abraxas in their study) as a BRCA1-interacting protein[15]. Similar to our current study, they also showed that CCDC98/Abraxas interacts with BRCA1 in a phosphorylation-dependent manner via its C-terminal SPTF motif[15]. Here, we have expanded on our initial observations and demonstrated a hierarchy in this DNA damage signal transduction pathway. We show that although RAP80 is required for formation of both CCDC98 and BRCA1 foci, CCDC98 is required for formation of only BRCA1 and not RAP80 foci. Moreover, abolishing BRCA1 does not affect either RAP80 or CCDC98 focus formation after DNA damage. Thus, we are able to delineate a signaling pathway from RAP80 to CCDC98 and then to BRCA1 (**Fig. 4e**).

Our study also permits a better understanding of CCDC98's activity as a mediator in this process. We show that the N terminus of CCDC98 is required for RAP80 binding, and its C-terminal phosphorylation motif is required for BRCA1 binding. In agreement with the notion that CCDC98 functions downstream of RAP80, only the N-terminal RAP80-binding domain of CCDC98 is important for its localization to foci after DNA damage. Putting all these studies together, we now have a better understanding of the mechanisms underlying the recruitment of BRCA1 to damaged DNA. RAP80 binds directly to the N terminus of CCDC98. This interaction is not phosphorylation dependent, but rather allows formation of a stable complex between RAP80 and CCDC98. After DNA damage, the RAP80–CCDC98 complex localizes to damage sites. RAP80's localization to foci depends on its UIM domain, which probably binds unidentified ubiquitinated proteins at DNA breaks. Through its C-terminal phosphorylation motif, CCDC98 then recruits BRCA1 to the DNA damage sites and regulates BRCA1-dependent checkpoint control.

The factors that act upstream of the RAP80–CCDC98 complex and recruit it to sites of DNA damage remain elusive. What we do know is that both γH2AX and MDC1 are upstream regulators and are required for the focus formation of many checkpoint proteins, including RAP80 and BRCA1. Because the localization of RAP80 seems to depend on the ability of its UIM domain to bind polyubiquitinated proteins, we speculate that there is at least one E3 ubiquitin ligase involved in this process. This unidentified E3 ligase may act after γH2AX and MDC1 to facilitate protein ubiquitination at sites of DNA damage, which would in turn serve as a signal to recruit RAP80–CCDC98 and BRCA1. The identification of this E3 ligase and its substrates at DNA breaks would provide further insight into the complex regulation of DNA damage response pathways.

Although RAP80 and CCDC98 seem to function upstream of BRCA1 in the DNA damage signal transduction pathway, it is noteworthy that checkpoint defects observed in *RAP80*- or *CCDC98*-deficient cells are not as severe as those observed in cells with a *BRCA1* deficiency. One likely explanation is that there are proteins other than RAP80 and CCDC98 that also participate in regulating BRCA1 function after DNA damage. We hope that future studies will identify this parallel pathway, revealing exactly how the tumor suppressor BRCA1 is regulated after DNA damage and contributes to the maintenance of genomic stability.

METHODS

Plasmids. Human *CCDC98* full-length complementary DNA was obtained using reverse-transcription PCR. Wild-type human CCDC98 and its point mutants and deletion mutants were generated by PCR and subcloned into a modified pIRES-EGFP mammalian expression vector to create constructs encoding SFB-tagged wild-type or mutant CCDC98. DNA fragments encoding BRCA1-BRCT domain and RAP80 were also generated by PCR and subcloned into pGEX-4T-1 vector (Pharmacia) to make constructs for expression of GST–BRCA1-BRCT and GST-RAP80, respectively. Myc-BRCA1, Myc-BRCA1ΔBRCT, and full-length human RAP80 and its deletion mutants were described[13].

Cell culture and treatment with ionizing radiation. HeLa, U2OS and 293T cells were purchased from the American Type Culture Collection and maintained in RPMI 1,640 medium supplemented with 10% (v/v) FBS at 37 °C in 5% CO_2. Cells were irradiated at the indicated doses using a JL Shepherd ^{137}Cs radiation source. The irradiated cells were then returned to the same culture conditions and maintained for the periods of time specified in the figure legends.

Short interfering RNA. All siRNA duplexes used in this study were purchased from Dharmacon. The sequences of *RAP80* siRNA, *CCDC98* siRNA 1, *CCDC98* siRNA 2, *BRCA1* siRNA and the control siRNA are 5′-GAAGGAUGUGGAAACUACCdTdT-3′, 5′-CAGGGUACCUUUAGUGGUUUU-3′, 5′-ACACAAGACAAACGAUCUAUU-3′ and 5′-UCACAGUGUCCUUUAUGUAdTdT-3′ and 5′-UUCAAUAAAUUCUUGAGGUUU-3′, respectively. siRNAs were transfected into the cells using Oligofectamine (Invitrogen) according to the manufacturer's instructions.

Antibodies, transfection and immunoprecipitation procedures. Rabbit antibodies to BRCA1, CCDC98 and RAP80 were raised by immunizing rabbits with GST-BRCA1 fragments, GST-CCDC98 and GST-RAP80 (residues 1–354) respectively. Phosphospecific antibody to Ser406 was generated by immunizing rabbits with KLH-conjugated GFGEYSR-pS-PTF peptide. The resulting rabbit polyclonal sera were affinity-purified using the SulfoLink or AminoLink Plus Immobilization and Purification Kit (Pierce). γH2AX antibodies were described[22]. Antibodies to Flag and β-actin were purchased from Sigma. Antibody to phosphorylated histone H3 (pH3) was purchased from Upstate Biotechnology. Transient transfection was done using Fugene 6 transfection reagent (Roche) according to the manufacturer's instructions. For immunoprecipitation, cells were washed with ice-cold PBS and then lysed in NETN buffer (0.5% (v/v) Nonidet P-40, 20 mM Tris (pH 8.0), 50 mM NaCl, 50 mM NaF, 100 μM Na_3VO_4, 1 mM DTT and 50 μg ml^{-1} PMSF) at 4 °C for 10 min. Crude lysates were cleared by centrifugation at 14,000 r.p.m. (Micro 240A, Scientific) and 4 °C for 5 min, and supernatants were incubated with protein A–agarose–conjugated primary antibodies. The immunocomplexes were washed three times with NETN buffer and then subjected to SDS-PAGE. Western blotting was done using the antibodies specified in the figures.

Cell lines and affinity purification of SFB-tagged protein complexes. To establish cell lines stably expressing various epitope-tagged proteins, 293T cells were transfected with plasmids encoding SFB-tagged proteins and pGK-Puro. Forty-eight hours after transfection, the cells were split at a 1:10 ratio and cultured in medium containing puromycin (10 μg ml^{-1}) for 3 weeks. The individual puromycin-resistant colonies were isolated and screened by western blotting. 293T cells stably expressing tagged proteins were lysed with 4 ml NETN buffer on ice for 10 min. Crude lysates were cleared by centrifugation at 14,000 r.p.m. (Micro 240A, Scientific) at 4 °C for 10 min, and supernatants were incubated with 300 μl streptavidin-conjugated beads (Amersham). The immunocomplexes were washed three times with NETN buffer and then bead-bound proteins were eluted with 500 μl NETN buffer containing 2 mg ml^{-1} biotin (Sigma). The eluted supernatant was incubated with 60 μl S protein beads (Novagen). The immunocomplexes were washed three times with NETN buffer and subjected to SDS-PAGE. Protein bands were visualized by silver staining, excised and digested, and the peptides were analyzed by mass spectrometry.

Glutathione *S*-transferase pull-down assay. GST fusion protein was expressed in *Escherichia coli* and purified as described[23]. GST fusion protein or GST alone (2 μg) was immobilized on glutathione-Sepharose 4B beads and incubated for 2 h at 4 °C with lysates prepared from cells transiently transfected with plasmids encoding the indicated proteins. After washing with NETN buffer, the samples were separated by SDS-PAGE and analyzed by western blotting.

Immunofluorescent staining. Cells grown on coverslips were fixed with 3% (w/v) paraformaldehyde at room temperature for 15 min and then permeabilized with PBS containing 0.5% (v/v) Triton X-100 at room temperature for 5 min. The coverslips were blocked with PBS containing 5% (v/v) goat serum at room temperature for 30 min before incubation with primary antibodies at room temperature for 20 min. After washing with PBS, cells were incubated with the secondary antibodies fluorescein isothiocyanate–conjugated goat anti-mouse IgG, rhodamine-conjugated goat anti-rabbit IgG or rhodamine-conjugated goat anti-mouse IgG (Jackson ImmunoResearch) at room temperature for 20 min. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI). After a final wash with PBS, coverslips were mounted with glycerin containing *p*-phenylenediamine. All images were obtained with a Nikon ECLIPSE E800 fluorescence microscope.

G2/M cell-cycle checkpoint assay. HeLa cells in a 100-mm plate were transfected twice with control or *CCDC98* siRNAs at 24-h intervals. Forty-eight hours after the second transfection, transfected cells were mock-treated or irradiated at indicated doses using a JL Shepherd ^{137}Cs radiation source. One hour after irradiation, cells were fixed with 70% (v/v) ethanol at –20 °C for 24 h, then stained with rabbit antibody to pH3 and incubated with fluorescein isothiocyanate–conjugated goat secondary antibody to rabbit immunoglobulin. The stained cells were treated with RNase A, incubated with propidium iodide and then analyzed by flow cytometry.

Cell survival assays. HeLa cells in a 60-mm plate were transfected twice with control or *CCDC98* siRNAs at 24-h intervals. Forty-eight hours after the second transfection, transfected cells were irradiated at the indicated doses using a JL Shepherd ^{137}Cs radiation source. Ten to twelve days after irradiation, cells were washed with PBS, fixed and stained with 2% (w/v) methylene blue, and the colonies were counted.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

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AUTHOR CONTRIBUTIONS

H.K., J.H. and J.C. designed experiments and interpreted the data; H.K. and J.H. performed all experiments; H.K. and J.C. prepared the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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