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hCds1 phosphorylates BRCA1 after DNA damage

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Keywords

ATM, BRCA1, CHK2, DNA damage, hCds1, phosphorylation, RAD53

Introduction

The hCds1 kinase, also known as CHK2, is activated in response to DNA damage and replication blocks. Activated hCds1 can induce checkpoints at both G1/S and G2/M in the cell cycle. The BRCA1 protein is involved in the homologous recombination-mediated repair of DNA double strand breaks. DNA damage during the S phase of the cell cycle results in the phosphorylation and dispersal of BRCA1 from the nuclear foci to which it is localised. Neither the significance nor the regulation of these DNA-damage-induced modifications of BRCA1 are fully understood.

Aims

To determine whether hCds1 directly phosphorylates BRCA1, and whether this affects the subsequent interactions between hCds1 and BRCA1 following irradiation of cells.

Comments

Ataxia-telangiectasia mutation (ATM) has previously been shown to be necessary for the phosphorylation of BRCA1 to occur in response to gamma-irradiation, and capable of directly phosphorylating BRCA1 *in vitro* (see additional information). This paper indicates that ATM can also cause the phosphorylation of BRCA1 by activating the hCds1/CHK2 kinase, for which BRCA1 is a substrate. Phosphorylation of BRCA1 in response to other genotoxins appears to be independent of ATM (Scully *et al*, *Cell* 1997, **90**: 425-435 [Abstract]). ATM-independent activation of hCds1/CHK2 may explain how some of these other genotoxins cause BRCA1 phosphorylation.

The ATM-hCds1/CHK2-BRCA1 DNA damage response pathway is clearly important for tumour suppression since heterozygous carriers of mutant *BRCA1*, *ATM* and *hCds1/hCHK2* genes (see additional information) have all been reported to be predisposed to breast cancer.

Methods

Phosphorylation of BRCA1 by hCds1 was analysed *in vitro* with kinase reactions using fragments of BRCA1 fused to glutathione-*S*-transferase (GST), and *in vivo* by using antibodies specific to Ser988-phosphorylated BRCA1. Subcellular localisation of BRCA1 and Cds1 was analysed by immunofluorescence, and BRCA1-hCds1 interaction was investigated using co-immunoprecipitation. The ability of mutant forms of BRCA1 to confer resistance to gamma irradiation was tested by gamma irradiating HCC1937 cells transfected with the mutant BRCA1 expression constructs.

Results

Recombinant hCds1 kinase was found to phosphorylate a GST-BRCA1 fusion protein containing amino acids 758-1064 of BRCA1.

Mass spectroscopy indicated that the GST-BRCA1 fusion protein was phosphorylated on amino Ser988 of BRCA1.

Phosphorylation of BRCA1 is induced by gamma irradiation in normal cells, but not in AT null cells, nor in cells expressing a kinase-dead hCds1.

Co-immunoprecipitation showed there is an interaction between BRCA1 and hCds1 before, but not after, gamma irradiation. However, gamma irradiation did not distinguish BRCA1 from kinase-dead hCds1, nor hCds1 from a mutant BRCA1 which could not be phosphorylated at Ser988 BRCA1 (S988A BRCA1).

Transfection of wild-type BRCA1 restored resistance to DNA damage within HCC1937 cells, which have mutant BRCA1. However, transfection of S988A BRCA1 did not confer DNA damage resistance.

Discussion

These results indicate that, in response to gamma irradiation, BRCA1 is phosphorylated on Ser988 by hCds1 directly. Phosphorylation of BRCA1 at Ser988 appears to regulate the interaction of BRCA1 with hCds1, and consequently the subcellular localisation of BRCA1. Importantly, phosphorylation at Ser988 seems to be necessary for BRCA1's role in the response to DNA damage, since S988A BRCA1, which cannot be phosphorylated at amino acid 988, does not rescue the DNA damage sensitivity of HCC1937 cells.

Additional information

BRCA1 has also been shown to be phosphorylated at serines 1423 and 1524 by ATM (Cortez *et al*, *Science* 1999, **285**: 1162-1166 [Abstract]) and at Ser1497 by CDK2-cyclin E during late G1 phase, and/or by CDK2-cyclin A during S phase (Ruffner *et al*, *Mol Cell Biol* 1999, **19**: 4843-4854 [Abstract]).

HCds1 is the human equivalent of *Saccharomyces cerevisiae* RAD53 and *Schizosaccharomyces pombe*Cds1.

Mutations in the hCds1/CHK2 gene have recently been shown to be responsible for the occurence of Li-Fraumeni syndrome in some families (Bell *et al*, *Science* 1999, **286**: 2528-2531 [Abstract]).

References

1. Lee J-S, Collins KM, Brown AL, Lee C-H, Chung JH: hCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response. Nature. 2000, 404: 201-204.