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BRCA1 and Rb?

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Keywords

BRCA1, cell cycle arrest, overexpression, Rb

Introduction

Germline mutation of *BRCA1* is found in a significant proportion of hereditary breast and ovarian cancers. A role for the BRCA1 protein as a negative regulator of cell growth has been suggested by two types of experiment. Firstly, overexpression of BRCA1 inhibits the growth of some cell lines. Secondly, attenuation of BRCA1 synthesis by antisense oligonucleotides can increase the proliferation rate of some cell lines.

Aims

To determine the mechanism of growth suppression by BRCA1 overexpression.

Comments

This paper, along with another recently published (see additional information), adds Rb to the ever expanding list of BRCA1-associated proteins. Furthermore, it suggests that BRCA1 is associated specifically with the hypophosphorylated active form of Rb which prevents cell cycle progression.

Overexpression of BRCA1 is shown to inhibit cell proliferation. This study demonstrates that this effect is Rb-dependent, while a previous study showed it to be $p21^{WAF1}$-dependent (Somasundaram *et al*: Nature, 1997, **389**: 187-190). Overexpression of BRCA1 can induce expression of $p21^{WAF1}$ which is thought to act upstream of Rb in inhibiting proliferation. Therefore the interaction of the BRCA1 and Rb proteins may not be involved in the growth arrest induced by BRCA1 overexpression. Whether such overexpression studies accurately reflect the physiological role of BRCA1 is not clear since loss of *Brca1* in mouse models also leads to proliferative arrest.

Methods

Growth suppression by BRCA1 was analysed by colony formation assay and BrdU incorporation. The interaction between BRCA1 and retinoblastoma (Rb) was investigated by GST pull-down assays of *in vitro*-translated proteins and cell lysates as well as by co-immunoprecipitation of the endogenous proteins from cell lysates.

Results

BRCA1 was overexpressed in a panel of cell lines, but only suppressed colony formation in those with an intact Rb gene. The requirement of Rb for this process was confirmed using fibroblasts from Rb mutant or wild type mouse embryos. Overexpression of BRCA1 decreased BrdU incorporation in Rb+/+ fibroblasts, but not in Rb-/- fibroblasts.

In vitro-translated BRCA1 was bound by GST-Rb fragments and *in vitro*-translated Rb was bound by GST-BRCA1 fragments. While BRCA1 fragments containing amino acids 1-394 and 304-772 bind GST-Rb, a BRCA1 fragment containing amino acids 1-303 does not. Overexpression of a BRCA1 mutant with an inframe deletion from amino acids 303-394 has no effect on colony formation.

Endogenous BRCA1 was co-immunoprecipitated with Rb antibodies and endogenous Rb was co-immunoprecipitated with a BRCA1 antibody from U2OS cell lysate. Only the hypophosphorylated (active) form of the Rb protein appeared to co-immunoprecipitate with the BRCA1 antibody.

Discussion

Although the presence of Rb may be required for BRCA1-mediated growth arrest, the interaction of these two proteins might not. Indeed, Rb acts as a downstream effector of several growth suppression pathways.

The complex containing Rb and histone deacetylase is thought to suppress transcription of E2F-responsive genes. Given the proposed role of BRCA1 in transcriptional regulation, one possible explanation for Rb-dependent BRCA1 growth suppression is that BRCA1 targets the Rb-histone deactylase complex to specific genes regulated by progression through the cell cycle.

If Rb is a modulator of BRCA1 action, it is conceivable that reduction in the level of Rb expression in breast epithelia may induce a BRCA1 'null' phenocopy in BRCA1 carriers with potentially reduced BRCA1 expression. If this were the case, factors augmenting Rb expression might serve to delay the onset of cancers in these susceptible women.

Additional information

Yarden and Brody (Proc Natl Acad Sci (USA) 1999, **96**: 4983-4988[Abstract]) have also recently published evidence for an interaction between BRCA1 and Rb. Using immunostaining they find that BRCA1 and Rb are colocalised in nuclear dots. They also demonstrate that GST-Rb can pull down *in vitro*-translated BRCA1 C-terminal domain (BRCT). Thus BRCA1 appears to have two Rb-binding sites, one in the C-terminal domain (Yarden and Brody) and one between amino acids 304 and 394 (Aprelikova *et al*). Interestingly, Rb itself contains a diverged version of the BRCT domain found in BRCA1 (Bork *et al*: FASEB J, 1997, **11**: 68-76[Abstract]).

References

1. Aprelikova ON, Fang BS, Meissner EG, Cotter S, Campbell M, Kuthiala A, Bessho M, Jensen RA, Liu ET: BRCA1-associated growth arrest is RB-dependent. Proc Natl Acad Sci USA. 1999, 96: 11866-11871.