EXPERIMENTAL CELL RESEARCH 316 (2010) 2017–2026

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Research Article

Upregulated Op18/stathmin activity causes chromosomal instability through a mechanism that evades the spindle assembly checkpoint

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ARTICLE INFORMATION

Article Chronology:
Received 12 March 2010
Revised version received
9 April 2010
Accepted 12 April 2010
Available online 25 April 2010

Keywords:
Aneuploidy
Micronucleus
Merotely
Merotelic
Phosphorylation
APC/C

ABSTRACT

Op18/stathmin (Op18) is a microtubule-destabilizing protein that is phosphorylation-inactivated during mitosis and its normal function is to govern tubulin subunit partitioning during interphase. Human tumors frequently overexpress Op18 and a tumor-associated Q18→E mutation has been identified that confers hyperactivity, destabilizes spindle microtubules, and causes mitotic aberrancies, polyploidization, and chromosome loss in K562 leukemia cells. Here we determined whether wild-type and mutant Op18 have the potential to cause chromosomal instability by some means other than interference with spindle assembly, and thereby bypassing the spindle assembly checkpoint. Our approach was based on Op18 derivatives with distinct temporal order of activity during mitosis, conferred either by differential phosphorylation inactivation or by anaphase-specific degradation through fusion with the destruction box of cyclin B1. We present evidence that excessive Op18 activity generates chromosomal instability through interference occurring subsequent to the metaphase-to-anaphase transition, which reduces the fidelity of chromosome segregation to spindle poles during anaphase. Similar to uncorrected merotelic attachment, this mechanism evades detection by the spindle assembly checkpoint and thus provides an additional route to chromosomal instability.

Introduction

The microtubule cytoskeleton is made up of α/β tubulin subunits and serves distinct specialized functions during interphase and mitosis [reviewed in 1]. Formation of the bipolar spindle at mitosis involves attachment of microtubules to kinetochores on the duplicated chromosomes [reviewed in 2]. Spindle assembly is surveyed by a checkpoint mechanism that senses unattached kinetochores and delays the transition from metaphase to anaphase until all chromosomes are appropriately connected to microtubules [reviewed in 3]. At the molecular level, the checkpoint acts by inhibiting the ubiquitin ligase activity of the anaphase-promoting complex (APC/C), which mediates destruction of proteins such as securin and cyclin B [reviewed in 4].

Complete inactivation of the spindle assembly checkpoint by specific mutations results in massive chromosome mis-segrega-

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Abbreviations: APC/C, anaphase-promoting complex; Op18, oncoprotein 18/stathmin; Op18-Q18E, Op18 in which Glu 18 is exchanged to Gln; Op18-tetraA, Op18 in which Ser-16, Ser-25, Ser-38, and Ser-63 are exchanged to Ala; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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doi:10.1016/j.yexcr.2010.04.008

tion and is lethal for mammalian cells after a few cell divisions [5]. However, construction of mouse models in which checkpoint signaling is only partially weakened has allowed generation of strains that develop normally but that contain cell populations with numerical alterations of whole chromosomes [6–8]. In general, these strains have much higher incidences of spontaneous and/or carcinogen-induced tumors. The relative levels of aneuploidy of mouse strains with weakened checkpoint signaling do not, however, correlate with the tumor incidence, which indicates that there is a complex interplay with other genetic defects that are required for malignant growth [reviewed in 9].

The majority of human tumors are aneuploid, i.e. they have an abnormal number of chromosomes. This probably reflects the fact that chromosomal instability is an important part of the genetic instability known to drive tumor progression [reviewed in 10]. In some cases, chromosomal instability can be attributed to weakened spindle assembly checkpoint signaling but the fidelity of chromosome segregation may also be reduced by other means, e.g. defects in (i) cohesion between sister chromatids, (ii) proteins involved in resolving merotelic kinetochore attachment before the onset of anaphase, and (iii) centrosome number through aberrant cytokinesis or mitotic slippage [reviewed in 9]. Thus, chromosomal instability may arise through many types of tumor-associated defects that perturb either the early or the late phases of mitosis.

Op18/stathmin (Op18) functions by mediating rapid changes in the density of the interphase microtubule array in response to stimulation of diverse signal-transducing kinase systems, which phosphorylate Op18 and thereby switch off its microtubule-destabilizing activity [reviewed in 11]. Op18 has been shown to act as a predominant regulator of tubulin monomer–polymer partitioning [12] and also microtubule nucleation at the centrosome during interphase [13]. As somatic mammalian cells enter mitosis, Op18 is inactivated by multisite phosphorylation by spindle assembly regulating kinase systems including CDK1, PLK1, and Aurora B [14–16]. This indicates that Op18 activity is switched off until the spindle assembly checkpoint is silenced, which is consistent with the apparently normal spindle assembly in both human and murine somatic cells lacking Op18 [13,17]. Thus, the physiologically relevant function of Op18 appears to be confined to the interphase of the cell cycle.

Op18 expression is frequently upregulated in a diverse array of tumors [reviewed in 18]. Moreover, a somatic mutation in the Op18 gene, which results in substitution of Gln for Glu at amino acid position 18 (termed Op18-Q18E), has been identified in a human adenocarcinoma and shown to confer oncogenic properties to the Op18 protein [19]. We have reported that the Op18-Q18E protein is hyperactive and only partially inactivated by phosphorylation at mitotic entry [17]. Accordingly, even modest Op18-Q18E expression markedly interferes with spindle assembly and produces a pronounced aneugenic effect, i.e. frequent mis-segregation of chromosomes that results in the formation of micronuclei. Under conditions of overexpression, wild-type Op18 also exerts an aneugenic effect, which we interpreted at that time as failure to phosphorylation-inactivate excessive amounts of Op18 during prophase of mitosis [17].

Our previous report was based on Op18-mediated aneugenic effects in the K562 leukemia cell line, which has a hypo-triploid karyotype and may have a weakened spindle checkpoint [20]. Here we address the mechanism(s) behind the Op18-mediated aneugenic effect in the pseudo-diploid Jurkat leukemia cell line, which appears to have a stable karyotype [21] and a robust spindle assembly checkpoint [22]. To our initial surprise, a robust checkpoint does not significantly protect against the aneugenic effect of low-level expression of Op18-Q18E. However, checkpoint evasion could be explained by the evidence presented here for a mechanism by which excessive Op18 activity perturbs the spindle subsequent to the metaphase-to-anaphase transition.

Materials and methods

DNA constructs

Construction of the pMEP4 shuttle vector directing inducible expression of Flag epitope-tagged wild-type Op18, Op18-Q18E (Gln-18 exchanged to Glu), or phosphorylation site-deficient Op18-tetraA (with Ser-16, Ser-25, Ser-38, and Ser-63 exchanged to Ala) has been described [17,23]. The Op18-Q18E-D box fusion derivative, which contains a sequence of cyclin B1 that confers APC/C-dependent proteosomal degradation of Op18-Q18E, was designed according to personal communications with Jonathon Pines, Gurdon Institute, Cambridge, UK. A DNA fragment corresponding to the N-terminal 90-amino acid sequence of cyclin B1 was prepared by PCR using a human cyclin B1 cDNA (accession number NM_031966, kindly provided by Jonathon Pines) as template with primers 5′-GGA CCC AAG CTT GTC GCT TGT CTT CTA TTC ACC ATG CTC CGA GTC ACC AGG AAC TCG-3′ and 5′-GCC GCA GCC ATG GCT CCC ACC AGC ATA GGT ACC TTT TCA AG-3′. The PCR fragment was digested with *Hind*III and *Nco*I and inserted between the corresponding sites of Op18-Q18E carried on pBluescript SK to create an N-terminal fusion to the initiator Met of Op18-Q18E. The coding sequence of the PCR-generated fragment was confirmed by nucleotide sequence analysis. For expression in cell lines, Op18-Q18E-D box encoding DNA was subcloned as a *Hind*III–*Bam*HI fragment into the EBV-based shuttle vector pMEP4 (Invitrogen).

Transfections and cell culture

Transfections of K562 and Jurkat cells using EBV-based replicating shuttle vectors and subsequent selection of hygromycin-resistant cell lines were performed as described in detail elsewhere [12]. For graded expression levels of ectopic Op18-Q18E, cells were transfected with 0, 4, 8, 12, or 16 μg of pMEP-Op18-Q18E mixed with the empty pMEP vector up to a total quantity of 16 μg DNA. Due to the stringent replication control of the EBV-based shuttle vectors, the ratio of transfected DNAs is stable during the 5- to 7-day time course of the present experiments [24]. Conditional expression was induced from the hMTIIa promoter of the pMEP vector by the addition of Cd^{2+} as detailed for K562 and Jurkat cells in [12].

Immunoblotting, quantification of tubulin monomer–polymer partitioning, and flow cytometry

Immunoblotting and subsequent detection using the ECL detection system (Amersham Pharmacia Biotech) were performed using anti-α-tubulin (B-5-1-2, Sigma) and affinity-purified rabbit antibodies raised against an internal peptide sequence of Op18, corresponding to residues 46–58 [25]. Quantification of cellular microtubule content in interphase cells, Op18 content, analysis of DNA content, and determination of the mitotic index by flow cytometry has been