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Alternative Splicing of *CHEK2* and Codeletion with *NF2* Promote Chromosomal Instability in Meningioma[1]

Hong Wei Yang*, Tae-Min Kim†, Sydney S. Song*, Nihal Shrinath*, Richard Park†, Michel Kalamarides‡,§, Peter J. Park†, Peter M. Black*, Rona S. Carroll* and Mark D. Johnson*

*Department of Neurosurgery, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA; †Center for Biomedical Informatics, Harvard Medical School, Boston, MA, USA; ‡Inserm, U674, Paris, France; §Université Paris 7 - Denis Diderot, Institut Universitaire d'Hématologie, Paris, France

Abstract

Mutations of the *NF2* gene on chromosome 22q are thought to initiate tumorigenesis in nearly 50% of meningiomas, and 22q deletion is the earliest and most frequent large-scale chromosomal abnormality observed in these tumors. In aggressive meningiomas, 22q deletions are generally accompanied by the presence of large-scale segmental abnormalities involving other chromosomes, but the reasons for this association are unknown. We find that large-scale chromosomal alterations accumulate during meningioma progression primarily in tumors harboring 22q deletions, suggesting 22q-associated chromosomal instability. Here we show frequent codeletion of the DNA repair and tumor suppressor gene, *CHEK2*, in combination with *NF2* on chromosome 22q in a majority of aggressive meningiomas. In addition, tumor-specific splicing of *CHEK2* in meningioma leads to decreased functional Chk2 protein expression. We show that enforced Chk2 knockdown in meningioma cells decreases DNA repair. Furthermore, Chk2 depletion increases centrosome amplification, thereby promoting chromosomal instability. Taken together, these data indicate that alternative splicing and frequent codeletion of *CHEK2* and *NF2* contribute to the genomic instability and associated development of aggressive biologic behavior in meningiomas.

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Introduction

Although most meningiomas grow slowly, 10% to 15% of these tumors are WHO grade 2 or 3 lesions that display increased growth and recurrence rates. Mutation or deletion of the *NF2* gene on chromosome 22q is observed in nearly half of all sporadic meningiomas [1], and germ line mutations of *NF2* (as observed in neurofibromatosis type 2) lead to the development of meningiomas in humans and in mice [2]. Monosomy 22q often occurs in the context of *NF2* mutation and is the earliest and most frequent chromosomal alteration observed in meningiomas [1,3,4]. We and others have shown that meningiomas that display deletions of chromosome 22q are more likely to display other large-scale chromosomal alterations [5]. The presence of frequent large-scale chromosomal alterations correlates with increasing histologic grade and the risk of recurrence in meningiomas [5–7].

Based on the association between chromosome 22q deletions and the presence of other large-scale chromosomal changes in meningiomas, we hypothesized that 22q loss leads to defects in the DNA homologous recombination (HR) or nonhomologous end joining pathways, thereby increasing the accumulation of chromosomal alterations and promoting tumor progression in meningiomas. *CHEK2* is a tumor suppressor gene on chromosome 22q that is located near the *NF2* gene and that encodes a kinase (Chk2) that is involved in the HR and nonhomologous end joining DNA repair pathways [8]. Here we show that alternative splicing

Address all correspondence to: Mark D. Johnson, MD, PhD, Department of Neurosurgery, Brigham and Women's Hospital, Boston, MA 02115. E-mail: mjohnson27@partners.org

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and frequent codeletion of *CHEK2* with *NF2* in meningiomas harboring chromosome 22q deletions impair DNA repair and increase chromosomal instability, thereby promoting meningioma progression.

Materials and Methods

Tumor Specimens and Cell Lines

All studies were performed with written informed consent and under the auspices of a human subjects institutional review board protocol approved by the Partners Human Research Committee. A total of 47 primary human meningioma specimens were used for whole genome analysis. Among these were 18 initial and recurrent pairs involving meningiomas that progressed to a higher histologic grade (17 patients with two specimens and 1 patient with three specimens) [5]. Four established human meningioma cell lines were used in this study (IOMM-Lee, CH157-MN, F5, and Me3TSC) [9–13]. CH157-MN was obtained from Dr DH Gutmann, Washington University School of Medicine, St. Louis, MO. All cell lines were cultured in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum for less than six passages and were maintained at 37°C in a 5% CO_2 atmosphere.

Reverse Transcription–Polymerase Chain Reaction, Cloning, and Direct Sequencing of CHEK2 *Transcripts*

Total RNA was extracted from primary meningioma specimens, and one-step reverse transcription–polymerase chain reaction (RT-PCR) for *CHEK2* was performed. The primers used were 5′-ATGTCTCGGGAGTCGGATG (sense) and 5′-ACCACGGAGTTCACAACACAG (antisense). The PCR products were separated on a 1.2% agarose gel to visualize alternatively spliced transcripts of *CHEK2*. The PCR products were then cloned into a pGEMT-Easy vector (Promega, Madison, WI), and 10 colonies were randomly selected for direct sequencing.

Lentivirus Production and Generation of Chk2 Knockdown Meningioma Cell Lines

Lentiviral small hairpin RNA (shRNA) vectors for *CHEK2* (clones V2LHS_1932 and V2LHS_196805) as well as a pGIPZ control vector were purchased from Open Biosystems (Rockford, IL). The lentiviral vectors were packaged in 293FT cells using the ViraPower Lentiviral Expression System (Invitrogen, Grand Island, NY) according to the manufacturer's protocol. Human IOMM-Lee and CH157-MN meningioma cells were transduced with the appropriate lentiviruses, and stable cell lines were selected using puromycin.

500K SNP and Genome-wide Array Comparative Genomic Hybridization Analyses

500K single nucleotide polymorphism (SNP) analysis of DNA extracted from primary human meningioma specimens was performed as described previously [5]. Briefly, the tumor cell content of each meningioma specimen was first evaluated histologically using hematoxylin and eosin, and only specimens with a tumor cell content greater than 90% were used for analysis. Genomic DNA was isolated using a commercially available kit (Qiagen, Valencia, CA). The DNA was then labeled and analyzed using Affymetrix 500K single nucleotide polymorphism (SNP; Santa Clara, CA) arrays according to the manufacturer's protocol. The data were normalized and analyzed using the Affymetrix GTS software package.

For the array comparative genomic hybridization (CGH) analysis, cultured IOMM-Lee cells stably expressing control or shChk2 vectors were exposed to a low, sublethal dose of UV irradiation (50 J/m^2, 1 minute) and then serially cultured for 10 passages. Meningioma cell genomic DNA was then extracted. Genomic test and reference DNA were independently labeled with fluorescent dyes, cohybridized to a NimbleGen Human CGH 385K Whole-Genome Tiling array (NimbleGen, Madison, WI) and scanned using a 5-μm scanner. Log_2 ratio values of the probe signal intensities (Cy3/Cy5) were calculated and plotted *versus* genomic position using Roche-NimbleGen NimbleScan software (NimbleGen) according to the manufacturer's protocol. The data were displayed using the Roche-NimbleGen SignalMap software (NimbleGen).

Cell Cycle Analysis

Established human CH157 meningioma cells were plated in 10-cm culture dishes for 24 hours. The cells were then labeled with propidium iodide, and flow cytometry cell cycle analysis was performed using a FACScan Flow Cytometer (BD Biosciences, Bedford, MA).

DNA Repair and Centrosome Duplication Assays

Cultured human meningioma cells were exposed to UV irradiation (50 J/m^2 for 5 minutes) to induce DNA double-strand breaks (DSBs). The cells were then fixed at various time points, and immunofluorescence was used to detect the presence of phospho-histone γ-H2AX foci as a marker of DSBs. Immunostaining was performed using an Alexa Fluor 488–conjugated phospho-histone γ-H2AX (Ser139) antibody (20E3; Cell Signaling Technology, Beverly, MA). The percentage of nuclei displaying γ-H2AX-immunoreactive foci was determined by direct counting.

Cultured meningioma cells or 293T cells expressing an sh*CHEK2* vector or a scrambled shRNA control vector were stained using an antipericentrin antibody (ab4448; Abcam, Cambridge, MA) to detect centrosomes. The number of centrosomes in each cell was determined by direct visualization under fluorescence microscopy. Centrosome number in at least 680 cells was determined for each condition. Cells containing three or more centrosomes were identified as abnormal. Statistical significance was determined using the proportion test.

Western Blots

Total protein was extracted using RIPA buffer supplemented with proteinase inhibitors and phosphatase inhibitor cocktail II (Boston Bioproducts, Ashland, MA). Protein extracts were then separated by gel electrophoresis using 10% SDS-PAGE–Tris-HCl gels. The protein was transferred to nitrocellulose membranes, washed, and subsequently probed using specific antibodies. Antibodies used included anti-Chk2 and anti-Cdc25A antibodies (Cell Signaling Technology), anti-NF2 (Abcam), or anti–β-Actin (Sigma) as a loading reference. After washing and incubating in the appropriate secondary antibody, immunoreactive bands were visualized using the enhanced chemiluminescence system (Pierce, Rockford, IL).

MTT Growth Assay

Meningioma cells (CH157-MN, IOMM-Lee, F5 or Me3TSC) were plated in 96 well plates (1×10^4 cells/well) and maintained in growth medium supplemented with serum. MTT fluorometric assays were performed according to the manufacturer's protocol as described previously [14]. Six wells were used for each condition. Statistical significance was determined using the *t* test.

Results

Alternative Splicing and Deletion of CHEK2 *in Meningioma*

To further investigate the relationship between 22q deletions, the accumulation of large-scale chromosomal abnormalities, and tumor progression, we performed a 500K SNP analysis of DNA from 18 initial (I) and recurrent (R) paired meningioma specimens where the recurrent tumor progressed to a higher histologic grade. As seen in Figure 1, tumors that lacked 22q deletions generally progressed to a higher grade without accumulating additional large segmental chromosomal changes. In one case, progression to a higher grade was accompanied by the new appearance of a 22q deletion concurrent with other segmental chromosomal changes (*arrow*, Figure 1*A*). Each of the remaining nine tumors harbored 22q deletions and displayed an increase in segmental chromosomal deletions during progression. The overall pattern of chromosomal changes that developed was similar to that reported in previous genomic studies of nonrecurrent meningiomas and included frequent losses of 22q, 1p, and 14q [4–7]. Thus, the accumulation of frequent segmental chromosomal changes was observed almost exclusively in tumors harboring deletions of chromosome 22q (Figure 1*A*). Tumors lacking segmental chromosome 22q deletions progressed without accumulating such changes. The accumulation of segmental chromosomal changes thus accompanies tumor progression and is closely associated with chromosome 22q loss in a majority of aggressive meningiomas.

The observed association between 22q deletion and the accumulation of segmental chromosomal abnormalities in meningiomas suggested the presence of a defect in pathways regulating chromosomal stability. We reasoned that this defect was likely to be present at the time of the earliest large-scale chromosomal changes and that it might

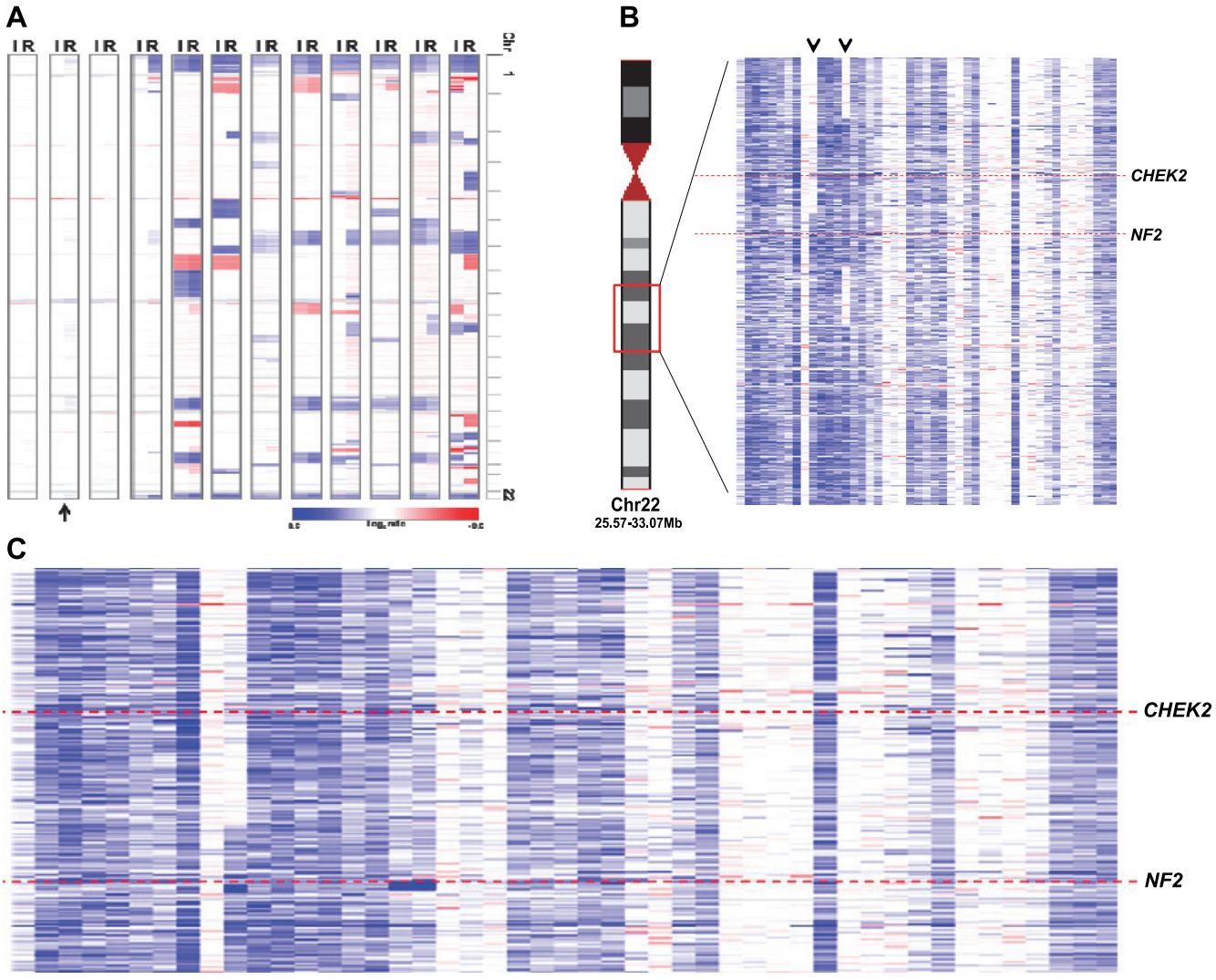
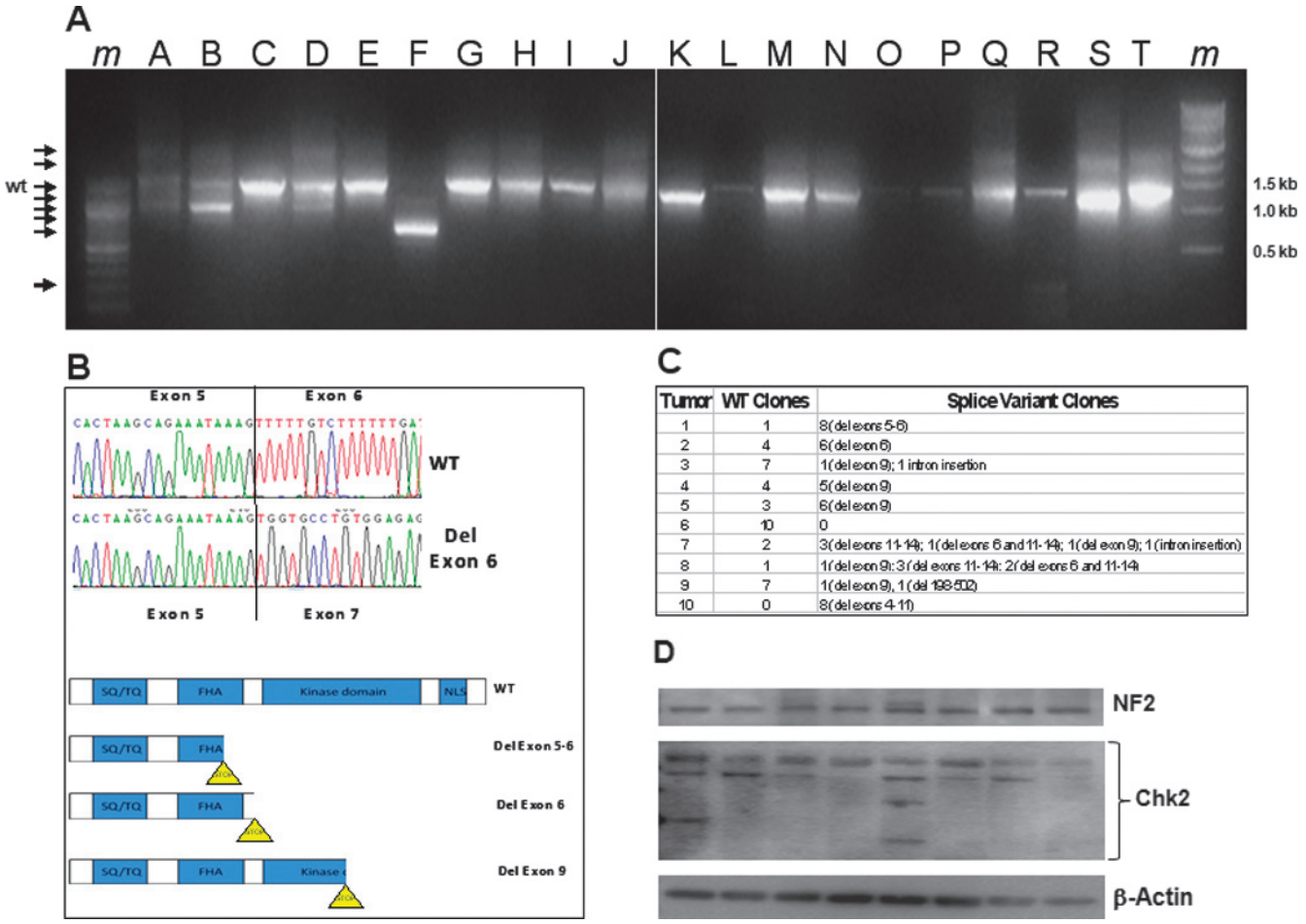


Figure 1. Chromosomal instability occurs in the context of 22q deletion during meningioma progression. (A) 500K SNP analyses for 12 initial (I) and recurrent (R) paired primary meningioma specimens illustrating accumulation of large-scale chromosomal changes at the time of recurrence. Note the association between 22q deletion and the presence of numerous segmental chromosomal abnormalities. Arrow identifies a pair of specimens in which the initial specimen lacked 22q deletion, whereas the recurrent tumor displayed 22q deletion and numerous additional large-scale chromosomal changes. (B) 500K SNP analyses of a portion of chromosome 22q in 47 human meningioma specimens. The location of the *CHEK2* and *NF2* genes is as indicated. Arrowheads point to two tumors with interstitial 22q deletions that involve *NF2* and *CHEK2*. (C) Higher-resolution image of the SNP data shown in B illustrating frequent codeletion of *NF2* and *CHEK2* in meningioma.



Tumor	WT Clones	Splice Variant Clones
1	1	8(del exons 5-6)
2	4	6(del exon 6)
3	7	1(del exon 9); 1 intron insertion
4	4	5(del exon 9)
5	3	6(del exon 9)
6	10	0
7	2	3(del exons 11-14); 1(del exons 6 and 11-14); 1(del exon 9); 1 (intron insertion)
8	1	1(del exon 9); 3 (del exons 11-14); 2(del exons 6 and 11-14)
9	7	1(del exon 9), 1 (del 198-502)
10	0	8(del exons 4-11)

Figure 2. Alternative splicing of *CHEK2* in meningioma yields nonfunctional *CHEK2* splice variants. (A) RT-PCR analysis of CHEK2 transcripts using total RNA extracted from 20 primary meningioma specimens (labeled A through T). Arrows indicate location of splice variants compared with the full-length CHEK2 mRNA (WT), M = marker. (B) Direct sequencing of CHEK2 clones illustrating the most commonly identified splice variants lacking the kinase domain. (C) Frequency of full-length (wt) CHEK2 clones *versus* various splice variants in 10 primary meningioma specimens. (D) Western blot illustrating Chk2 and NF2 protein expression in eight primary meningioma specimens. Note that in half of the specimens, alternate Chk2 isoforms were more abundant than full-length Chk2 (top band).

thus result from deletions involving chromosome 22q. To investigate this possibility, we examined chromosome 22q for copy number alterations involving genes that are involved in HR or DNA repair. We observed that the *CHEK2* tumor suppressor gene, which is located within 1.1 Mb of *NF2*, was codeleted with *NF2* in all 30 specimens harboring 22q deletions. Although most of these specimens displayed monosomy 22q, two displayed interstitial deletions that involved both *CHEK2* and *NF2* (Figure 1, *B* and *C*).

CHEK2 is a tumor suppressor gene that is involved in DNA repair and genome stability [15–17]. Loss of *CHEK2* has been associated with the development of breast, prostate, and colon cancer [8,18,19]. Thus, the high frequency of codeletion of *CHEK2* with *NF2* raised the possibility that altered *CHEK2* expression might contribute to the genomic instability observed in meningiomas harboring chromosome 22q deletions.

To examine the status of *CHEK2* in meningiomas more closely, we isolated mRNA from 20 primary human meningioma specimens and performed RT-PCR for *CHEK2*. Thirteen of the 20 tumors showed evidence for multiple splice variants of *CHEK2*. Importantly, full-length *CHEK2* mRNA was reduced or undetectable in 15 of 20 meningiomas (Figure 2*A*). To investigate this phenomenon further, we cloned and sequenced *CHEK2* transcripts from 10 primary meningiomas. About 7 to 10 randomly selected *CHEK2* clones were sequenced for each tumor to obtain an estimate of the relative abundance of the different *CHEK2* transcripts. Point mutations in the coding sequence of *CHEK2* were not identified. However, several novel *CHEK2* splice variants in which a frame shift introduced a stop codon and eliminated the kinase domain were observed (Figure 2*B*). The ratio of wild-type (WT) to alternative transcripts ranged from 10:0 to 1:8 (Figure 2*C*). Some tumors primarily expressed full-length *CHEK2* transcripts, whereas others expressed truncated, nonfunctional *CHEK2* splice variants lacking the kinase domain. Previous studies indicate that proteins derived from such splice variants dimerize with WT Chk2 and act as dominant negative proteins [20,21].

Western blot analysis using protein from eight primary meningiomas confirmed the presence of multiple isoforms of Chk2 protein in six of eight tumors (Figure 2*D*). In half of the cases, these alternate Chk2 isoforms were more abundant than full-length Chk2. Taken together, these data indicate that heterozygous deletion and alternative splicing of *CHEK2* occurs frequently in primary meningiomas. These genetic and posttranscriptional alterations are associated with decreased expression of full-length Chk2 protein.

Chk2 Depletion Decreases DNA Repair Capacity in Meningioma Cells

We next examined the effect of decreased expression of full-length Chk2 on DNA repair in meningioma cells. We performed SNP analyses of four established meningioma cell lines (CH157-MN, F5, IOMM-Lee, and Me3TSC). Two of these cell lines (CH157-MN and Me3TSC) showed 22q deletions involving both *NF2* and *CHEK2* (Figure 3*A*). These two cell lines also displayed numerous segmental chromosomal deletions, suggesting impaired DNA repair and chromosomal instability. To investigate this possibility, we exposed cells from each of the four meningioma cell lines to UV irradiation (50 J/m^2 for 5 minutes) to induce DNA DSBs. The cells were then fixed at several time points after irradiation and stained for phosphorylated gamma H2AX (γ-H2AX), a sensitive marker for DNA double strand breaks [22]. The two cell lines with 22q deletions involving *CHEK2* (CH157-MN and Me3TSC) showed significantly greater γ-H2AX immunoreactivity 3 hours after UV irradiation when compared with the cell lines in which *CHEK2* was intact (F5 and IOMM-Lee), indicating delayed DNA repair (Figure 3, *B* and *C*; $P < .05$, unpaired *t* test).

Chk2 is phosphorylated by ATM after DNA damage and facilitates DNA repair by phosphorylating and stabilizing p53, thereby inducing G_1 arrest [23]. Chk2 also cooperates with PML to mediate p53-independent apoptosis after radiation exposure [24]. In addition to its effects on p53, Chk2 phosphorylates the Cdc25A phosphatase to inhibit cell cycle progression [16]. We therefore examined the effect of Chk2 depletion on meningioma cell growth after DNA damage. First, we overexpressed two different shRNAs directed against *CHEK2* mRNA to knockdown Chk2 expression in human

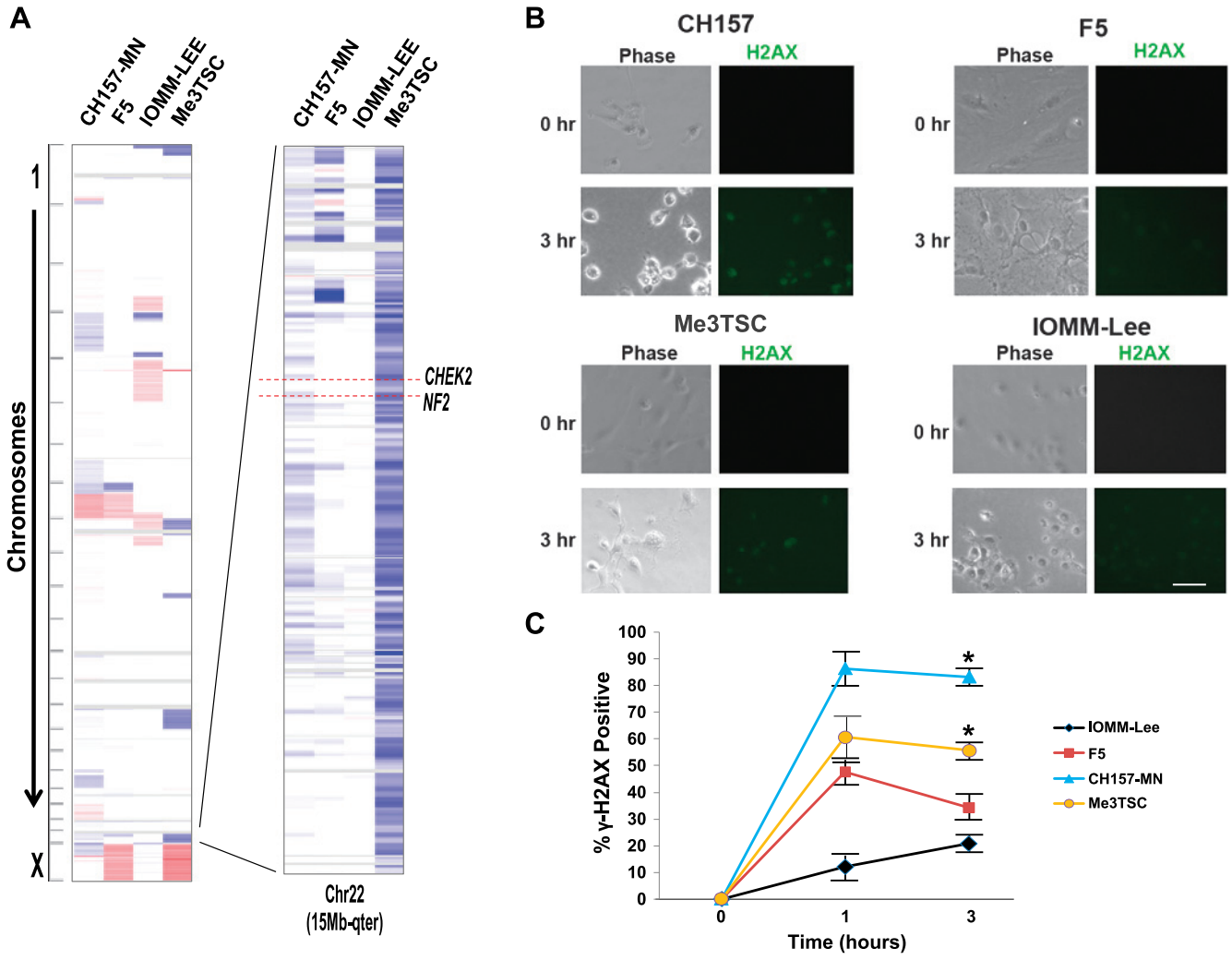


Figure 3. Impaired DNA repair capacity in meningioma cell lines with segmental 22q deletions. (A) 500K SNP analysis of genomic DNA obtained from four established human meningioma cell lines (CH157-MN, F5, IOMM-Lee, and Me3TSC). Dark blue represents copy number loss, whereas bright red represents gain. Each column contains data from a different cell line. Chromosome 22q is shown at higher magnification to the right. The approximate locations of the *NF2* and *CHEK2* genes are as indicated. Note that 22q deletions involving *CHEK2* and *NF2* occur in the CH157-MN and Me3TSC cell lines, but not the F5 and IOMM-Lee cell lines. (B) Cells from each of four human meningioma cell lines were exposed to ultraviolet irradiation (50 J/m^2) for 5 minutes. The cells were then fixed and stained for γ-H2AX immunoreactivity (green) after 0, 1, 2, and 3 hours to detect DNA DSBs. Note the relative increase in nuclear γ-H2AX immunoreactivity in the CH157-MN and Me3TSC cell lines that harbor *CHEK2* and *NF2* deletions when compared with the cell lines that do not have such deletions (F5 and IOMM-Lee). (C) Quantitation of data shown in B. A significant increase in γ-H2AX immunoreactivity was noted after 3 hours in human meningioma cell lines harboring *CHEK2* and *NF2* deletions (CH157-MN and Me3TSC) when compared with meningioma cell lines that do not have such deletions (F5 and IOMM-Lee). Data shown are mean ± SEM. **P* < .05, *t* test.

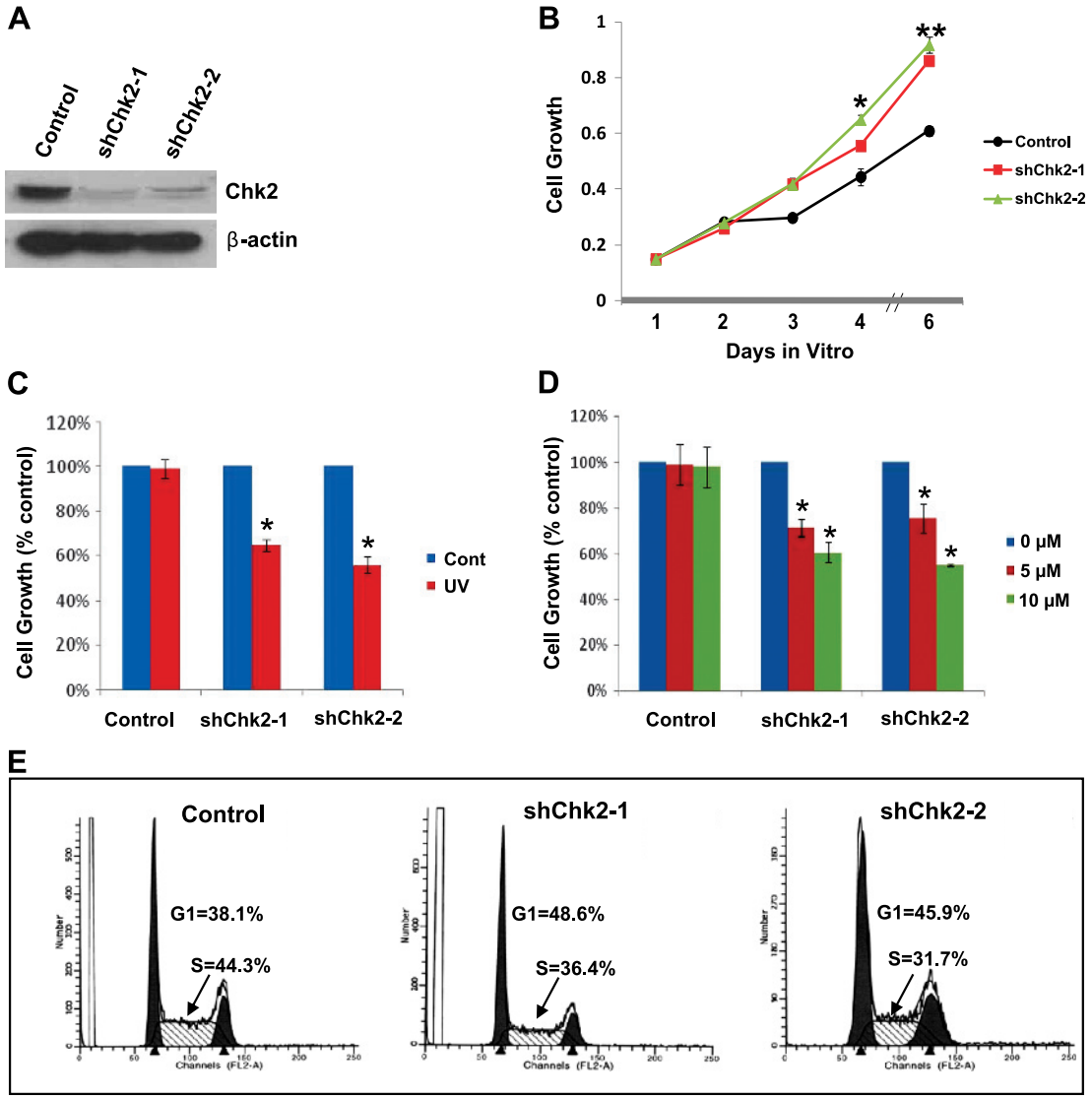


Figure 4. Chk2 depletion increases proliferation but decreases cell growth after DNA damage. (A) Western blot illustrating knockdown of Chk2 protein in CH157-MN cells stably expressing two different shRNAs directed against human Chk2. Control cells were transfected using an empty shRNA control vector. (B) MTT cell growth assay illustrating the effect of Chk2 depletion on the growth of human CH157-MN meningioma cells under control conditions. Data shown are mean ± SEM. *$P < .05$, **$P < .0001$, unpaired t test. (C) MTT cell growth assay illustrating effect of Chk2 depletion on the growth of CH157-MN cells after UV irradiation (50 J/m^2 for 5 minutes). Data shown are mean ± SEM. *$P < .05$, t test. (D) MTT cell growth assay illustrating effect of Chk2 depletion on the growth of CH157-MN cells after overnight exposure to increasing concentrations of camptothecin. Data shown are mean ± SEM. *$P < .05$, t test. (E) Flow cytometry cell cycle analysis illustrating effect of Chk2 depletion on CH157-MN cell proliferation.

CH157-MN meningioma cells. An 80% knockdown of Chk2 was achieved using this approach (Figure 4*A*). Cell growth assays revealed that Chk2 knockdown increased the growth of human CH157-MN meningioma cells under control conditions (Figure 4*B*; $P < .0001$, unpaired t test). This is consistent with the known role of Chk2 as a regulator of the G_2/M checkpoint [25]. Next, we measured the growth of these cells under control conditions or after UV irradiation. As shown in Figure 4*C*, depletion of Chk2 significantly decreased meningioma cell growth after UV irradiation-induced DNA damage ($P < .01$, unpaired t test). In addition, Chk2 depletion significantly decreased cell growth after DNA damage caused by exposure to the topoisomerase I inhibitor, camptothecin (Figure 4*D*; $P < .02$, unpaired t test). Similar effects of Chk2 knockdown on cell growth after UV irradiation or camptothecin-induced DNA damage were obtained using the IOMM-Lee meningioma cell line (data not shown).

To determine whether decreased expression of Chk2 alters cell cycle progression in meningioma, we knocked down Chk2 in CH157-MN meningioma cells and performed a cell cycle analysis using flow cytometry. Chk2 depletion decreased the number of cells in S phase and increased the fraction of cells in G_1 (Figure 4*E*). Taken together, these data demonstrate impaired DNA repair, increased sensitivity to DNA damage, and decreased proliferation in meningioma cells after Chk2 depletion.

Chk2 Depletion Promotes Centrosome Amplification and Chromosomal Instability

The striking accumulation of segmental chromosomal alterations observed in meningiomas suggests the presence of an abnormality in

pathways governing chromosomal stability. Centrosome amplification is a major contributor to chromosomal instability in cancer [26]. Previous studies have identified Cdc25A as a primary regulator of centrosome number [27], and Cdc25A is a direct target of Chk2 [16]. Chk2 phosphorylation of Cdc25A promotes its degradation [16]. We therefore investigated whether decreased Chk2 expression leads to genomic instability through centrosome amplification in meningioma cells.

Analysis of Chk2 and Cdc25A levels in a panel of 13 primary human meningiomas revealed an inverse correlation between full-length Chk2 expression and Cdc25A expression (Figure 5*A*). Additional Western blot analysis confirmed that enforced overexpression of Chk2 decreased Cdc25A levels, whereas overexpression of two different Chk2 splice variants lacking the kinase domain (cloned from primary meningioma specimens) failed to do so (Figure 5*B*). Immunocytochemistry for the centrosome marker, pericentrin, in 293T cells or CH157-MN meningioma cells revealed that Chk2 depletion increased centrosome number (Figure 5*C*). Quantitation of this effect indicated that the number of cells displaying three or more centrosomes increased two-fold after Chk2 knockdown (Figure 5*D*; $P < .011$, proportion test).

To determine whether the centrosome amplification occurring after Chk2 depletion was accompanied by an increased frequency of chromosomal alterations in meningioma cells, we administered a sublethal dose of UV irradiation (50 J/m^2 for 1 minute) to IOMM-Lee meningioma cells expressing a Chk2 shRNA vector or a control vector and maintained the cells for 10 passages in culture. Our previous SNP analysis indicated that IOMM-Lee cells did not harbor *CHEK2* deletions and were thus well suited for investigating the effects of Chk2 depletion on meningioma cells. After 10 passages, genomic DNA was extracted,

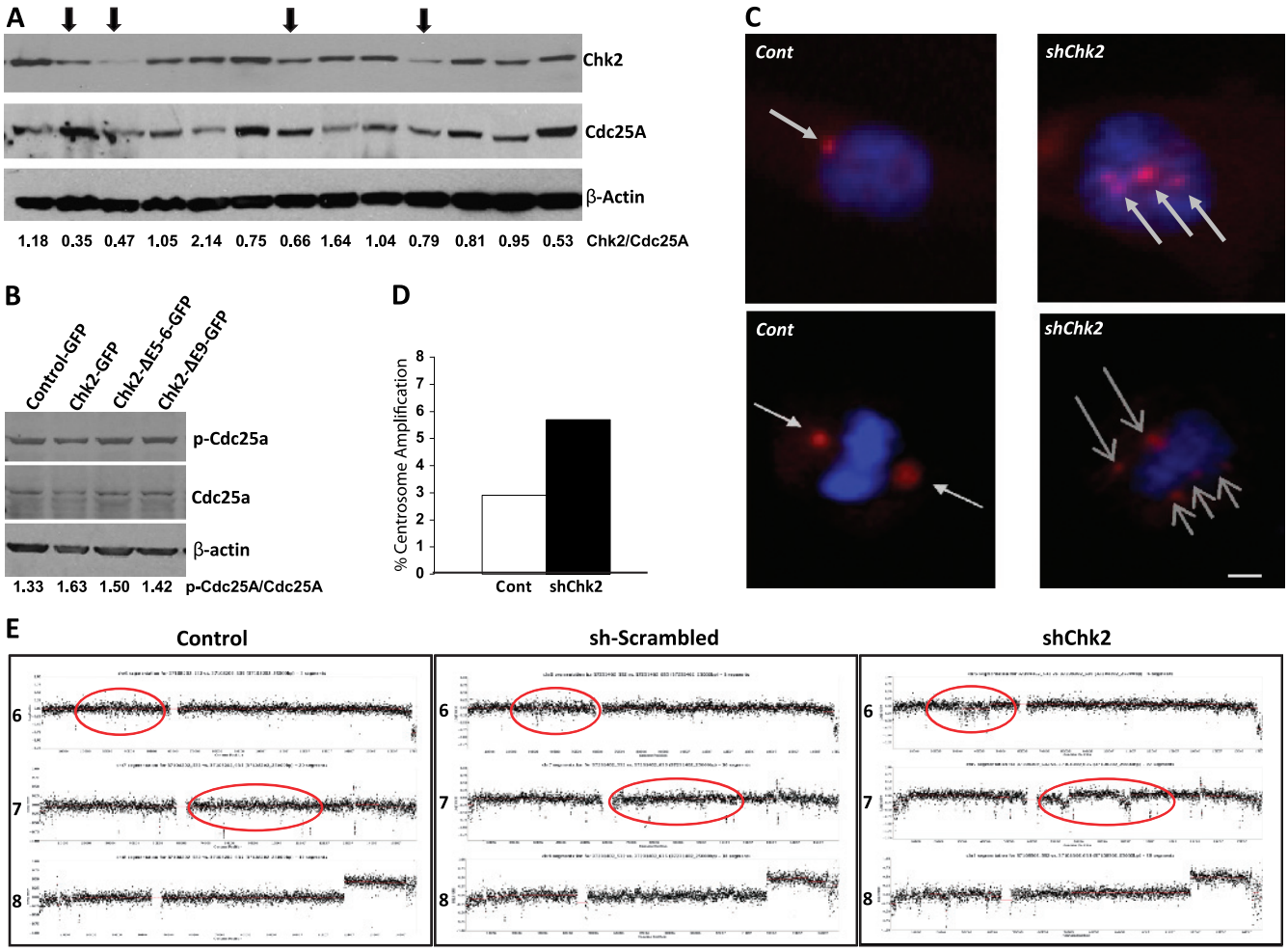


Figure 5. Chk2 depletion increases centrosome number and chromosomal instability in human meningioma cells. (A) Western blot illustrating correlation between Chk2 and Cdc25A expression using protein lysates derived from 13 primary human meningioma specimens. Arrows indicate samples with the lowest Chk2 expression. Densitometry measurements of the Chk2/Cdc25A ratio revealed a relative increase in Cdc25A expression in meningiomas with the lowest Chk2 levels. (B) Western blot illustrating effect of overexpressed Chk2 or Chk2 splice variants lacking the kinase domain on Cdc25A expression in 293T cells. Densitometry measurements revealed an increase in the p-Cdc25A/Cdc25A ratio and a decrease in the overall expression of Cdc25A after Chk2 overexpression. (C) Light micrographs illustrating effect of Chk2 depletion on centrosome number in IOMM-Lee meningioma cells. (D) Quantitative analysis of centrosome duplication induced by Chk2 depletion in CH157-MN meningioma cells. Cells with three or more centrosomes were considered abnormal. At least 680 cells were counted for each condition. $P < .011$, proportion test. (E) Array CGH analysis of DNA extracted from IOMM-Lee meningioma cells after Chk2 depletion. Cells were exposed to a sublethal dose of UV irradiation (50 J/m^2 for 1 minute) and were then passaged 10 times before harvesting. Data for chromosomes 6, 7, and 8 are shown. Note the increase of chromosomal deletions in cells with shChk2 depletion.

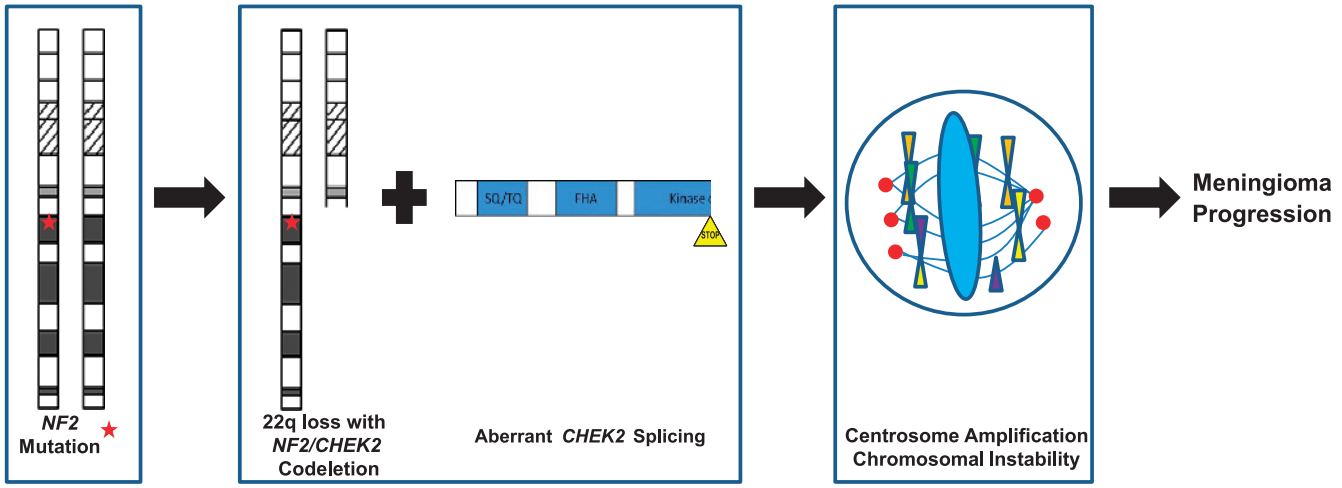


Figure 6. A proposed model by which a monoallelic *NF2* mutation prompts loss of the other *NF2* allele through 22q deletion. Monoallelic mutation of *NF2* leads to loss of the other *NF2* allele through segmental 22q deletion. This results in frequent codeletion of *CHEK2*, which is located close to the *NF2* gene. Concurrently, alternative splicing of *CHEK2* generates nonfunctional and dominant negative forms of Chk2. The combined effect of these changes leads to decreased Chk2 function, increased centrosome amplification, and chromosomal instability.

and array comparative genomic hybridization analysis was performed. As shown in Figure 5*E*, an increase in chromosomal alterations was observed in meningioma cells overexpressing Chk2 shRNA, but not in the parental meningioma cell line or in meningioma cells expressing a scrambled control shRNA.

Based on these findings, we propose a model (Figure 6) in which a monoallelic *NF2* mutation prompts loss of the other *NF2* allele through 22q deletion. This step initiates meningioma growth. Because of the close physical proximity of *CHEK2* to *NF2*, both genes are frequently lost when 22q is deleted. In addition, *CHEK2* is alternatively spliced in many meningiomas, leading to the production of nonfunctional or dominant negative forms of the kinase. This deletion and/or alternative splicing of *CHEK2* leads to decreased functional Chk2 protein, decreased DNA repair capacity, increased centrosome number, increased chromosomal instability, and consequent tumor progression.

Discussion

Here we provide evidence that alternative splicing and deletion of the tumor suppressor, *CHEK2*, contributes to chromosomal instability in meningioma. Our finding that deletion and decreased expression of functional Chk2 protein occurs in association with chromosome 22q deletions helps to explain the association between *NF2* mutations, 22q loss, and the occurrence of large-scale chromosomal deletions in meningioma. Monosomy 22q is found in 47% of meningiomas, and it occurs in all three histologic grades [1]. However, 22q deletions are observed with increased frequency in grade 2 and 3 meningiomas. As reported in other studies [1,4,6], we observed a general increase in the frequency of chromosomal abnormalities among a majority of clinically aggressive meningiomas, with a notable increase in the frequency of 22q, 1p, and 14q losses. Monosomy 22q is the earliest and most frequently occurring chromosomal abnormality noted in meningiomas, and its occurrence is driven by loss of heterozygosity of the *NF2* gene on 22q in most (but not all) of these tumors [1,28]. Importantly, the increased frequency of chromosomal alterations occurred primarily in tumors with deletions involving 22q. In a few cases, these alterations were interstitial deletions, but they invariably involved both *NF2* and *CHEK2*. The overall pattern suggests a model in which the accumulation of chromosomal changes accompanies tumor progression, and our analysis of initial and recurrent meningioma pairs clearly establishes that this is the case.

Several lines of evidence indicate that *CHEK2* is a bona fide tumor suppressor gene in humans. Germ line mutations in the *CHEK2* gene have been identified in a subgroup of families with Li-Fraumeni-like syndrome [15,29] and in patients with breast, prostate, or colon cancer [18–20]. Moreover, alternative splicing of *CHEK2* in breast or other cancers leads to the production of dominant negative Chk2 variants that can interfere with Chk2 function [20]. Our own data also indicate that several of the alternative splice variants of *CHEK2* found in meningioma lack the kinase domain and do not induce phosphorylation-dependent degradation of Cdc25a. Thus, a combination of *CHEK2* deletion and alternative splicing of *CHEK2* transcripts leads to decreased functional Chk2 expression in meningiomas.

The mechanisms by which loss of Chk2 promotes tumor formation are still being unraveled. After DNA damage, Chk2 stabilizes p53 to increase apoptosis [23] and promotes Cdc25A degradation to inhibit cell cycle progression [16]. Previous studies have suggested that Chk2 suppresses the oncogenic effect of DNA damage through these mechanisms [30]. It seems likely, however, that defects in DNA replication also contribute to the large-scale chromosomal instability observed in clinically aggressive meningiomas. Previous reports have suggested that inhibition of Chk2 activity can lead to p53-independent apoptosis through the induction of mitotic catastrophe [25]. Here we provide evidence that decreased Chk2 expression impairs DNA repair and contributes to chromosomal instability by increasing centrosome number. The loss of functional Chk2 protein increases centrosome number by increasing Cdc25a expression, which, in turn, regulates centrosome amplification [31]. In addition, loss of Chk2-dependent phosphorylation of BRCA1 may lead to abnormal mitotic spindle assembly, lagging chromosomes, and genomic instability [32]. Centrosome amplification alone can lead to chromosome misalignment and chromosomal instability [33], a common feature of clinically aggressive meningiomas.

Evidence from a mouse model of meningioma development suggests that focal *NF2* deletion alone leads to slowly growing benign tumors [2]. In patients who have neurofibromatosis II, most of the meningiomas detected are also slowly growing lesions, although aggressive tumors can develop. We have previously reported that recurrent alterations involving the CDKN2A/CDKN2B locus accompany meningioma progression from grade 2 to 3, but not from grade 1 to 2. Moreover, experiments in a mouse model of NF2-induced meningioma suggest that alterations to the CDKN2A/CDKN2B locus alone do not alter meningioma grade [5]. Presumably, CDKN2A/CDKN2B deletions cooperate with other accumulated chromosomal abnormalities to promote meningioma progression. It is important to note that we and other investigators have observed a subgroup of meningiomas with monosomy 22q and no other large-scale chromosomal abnormalities [4,5,34]. Unlike meningiomas with numerous large-scale chromosomal changes, tumors with monosomy 22q alone generally exhibit benign clinical behavior. It will be important to examine the status of *CHEK2* splice variants and overall Chk2 expression levels in this subset of meningiomas.

Taken together, our findings indicate that alternative splicing of *CHEK2* transcripts and codeletion of *CHEK2* with *NF2* impair DNA repair and promote chromosomal instability in meningioma. The resultant accumulation of large-scale chromosomal alterations contributes to the development of aggressive behavior in these tumors.

References

[1] Hansson CM, Buckley PG, Grigelioniene G, Piotrowski A, Hellstrom AR, Mantripragada K, Jarbo C, Mathiesen T, and Dumanski JP (2007). Comprehensive genetic and epigenetic analysis of sporadic meningioma for macro-mutations on 22q and micro-mutations within the NF2 locus. *BMC Genomics* **8**, 16.

[2] Kalamarides M, Niwa-Kawakita M, Leblois H, Abramowski V, Perricaudet M, Janin A, Thomas G, Gutmann DH, and Giovannini M (2002). *Nf2* gene inactivation in arachnoidal cells is rate-limiting for meningioma development in the mouse. *Genes Dev* **16**, 1060–1065.

[3] Lekanne Deprez RH, Bianchi AB, Groen NA, Seizinger BR, Hagemeijer A, van Drunen E, Bootsma D, Koper JW, Avezaat CJ, Kley N, et al. (1994). Frequent *NF2* gene transcript mutations in sporadic meningiomas and vestibular schwannomas. *Am J Hum Genet* **54**, 1022–1029.

[4] Arslantas A, Artan S, Oner U, Durmaz R, Muslumanoglu H, Atasoy MA, Basaran N, and Tel E (2002). Comparative genomic hybridization analysis of genomic alterations in benign, atypical and anaplastic meningiomas. *Acta Neurol Belg* **102**, 53–62.

[5] Goutagny S, Yang HW, Zucman-Rossi J, Chan J, Dreyfuss JM, Park PJ, Black PM, Giovannini M, Carroll RS, and Kalamarides M (2010). Genomic profiling reveals alternative genetic pathways of meningioma malignant progression dependent on the underlying *NF2* status. *Clin Cancer Res* **16**, 4155–4164.

[6] Weber RG, Bostrom J, Wolter M, Baudis M, Collins VP, Reifenberger G, and Lichter P (1997). Analysis of genomic alterations in benign, atypical, and anaplastic meningiomas: toward a genetic model of meningioma progression. *Proc Natl Acad Sci USA* **94**, 14719–14724.

[7] Lamszus K, Kluwe L, Matschke J, Meissner H, Laas R, and Westphal M (1999). Allelic losses at 1p, 9q, 10q, 14q, and 22q in the progression of aggressive meningiomas and undifferentiated meningeal sarcomas. *Cancer Genet Cytogenet* **110**, 103–110.

[8] Antoni L, Sodha N, Collins I, and Garrett MD (2007). CHK2 kinase: cancer susceptibility and cancer therapy—two sides of the same coin? *Nat Rev Cancer* **7**, 925–936.

[9] Yazaki T, Manz HJ, Rabkin SD, and Martuza RL (1995). Treatment of human malignant meningiomas by G207, a replication-competent multimutated herpes simplex virus 1. *Cancer Res* **55**, 4752–4756.

[10] Ragel BT, Jensen RL, Gillespie DL, Prescott SM, and Couldwell WT (2007). Celecoxib inhibits meningioma tumor growth in a mouse xenograft model. *Cancer* **109**, 588–597.

[11] Cargioli TG, Ugur HC, Ramakrishna N, Chan J, Black PM, and Carroll RS (2007). Establishment of an *in vivo* meningioma model with human telomerase reverse transcriptase. *Neurosurgery* **60**, 750–759, discussion 759–760.

[12] Lee WH (1990). Characterization of a newly established malignant meningioma cell line of the human brain: IOMM-Lee. *Neurosurgery* **27**, 389–395, discussion 396.

[13] Tsai JC, Goldman CK, and Gillespie GY (1995). Vascular endothelial growth factor in human glioma cell lines: induced secretion by EGF, PDGF-BB, and bFGF. *J Neurosurg* **82**, 864–873.

[14] Yang HW, Menon LG, Black PM, Carroll RS, and Johnson MD (2010). SNAI2/Slug promotes growth and invasion in human gliomas. *BMC Cancer* **10**, 301.

[15] Bell DW, Wahrer DC, Kang DH, MacMahon MS, FitzGerald MG, Ishioka C, Isselbacher KJ, Krainer M, and Haber DA (1999). Common nonsense mutations in RAD52. *Cancer Res* **59**, 3883–3888.

[16] Falck J, Lukas C, Protopopova M, Lukas J, Selivanova G, and Bartek J (2001). Functional impact of concomitant *versus* alternative defects in the Chk2-p53 tumour suppressor pathway. *Oncogene* **20**, 5503–5510.

[17] Chen Y and Poon RY (2008). The multiple checkpoint functions of CHK1 and CHK2 in maintenance of genome stability. *Front Biosci* **13**, 5016–5029.

[18] Dong X, Wang L, Taniguchi K, Wang X, Cunningham JM, McDonnell SK, Qian C, Marks AF, Slager SL, Peterson BJ, et al. (2003). Mutations in CHEK2 associated with prostate cancer risk. *Am J Hum Genet* **72**, 270–280.

[19] Kilpivaara O, Alhopuro P, Vahteristo P, Aaltonen LA, and Nevanlinna H (2006). CHEK2 I157T associates with familial and sporadic colorectal cancer. *J Med Genet* **43**, e34.

[20] Staalesen V, Falck J, Geisler S, Bartkova J, Borresen-Dale AL, Lukas J, Lillehaug JR, Bartek J, and Lonning PE (2004). Alternative splicing and mutation status of CHEK2 in stage III breast cancer. *Oncogene* **23**, 8535–8544.

[21] Lemaire M, Prime J, Ducommun B, and Bugler B (2004). Evolutionary conservation of a novel splice variant of the Cds1/CHK2 checkpoint kinase restricted to its regulatory domain. *Cell Cycle* **3**, 1267–1270.

[22] Schwenzer NF, Bantleon R, Maurer B, Kehlbach R, Schraml C, Claussen CD, and Rodegerdts E (2007). Detection of DNA double-strand breaks using γH2AX after MRI exposure at 3 tesla: an *in vitro* study. *J Magn Reson Imaging* **26**, 1308–1314.

[23] Chehab NH, Malikzay A, Appel M, and Halazonetis TD (2000). Chk2/hCds1 functions as a DNA damage checkpoint in G(1) by stabilizing p53. *Genes Dev* **14**, 278–288.

[24] Yang S, Kuo C, Bisi JE, and Kim MK (2002). PML-dependent apoptosis after DNA damage is regulated by the checkpoint kinase hCds1/Chk2. *Nat Cell Biol* **4**, 865–870.

[25] Castedo M, Perfettini JL, Roumier T, Yakushijin K, Horne D, Medema R, and Kroemer G (2004). The cell cycle checkpoint kinase Chk2 is a negative regulator of mitotic catastrophe. *Oncogene* **23**, 4353–4361.

[26] Lingle WL, Lukasiewicz K, and Salisbury JL (2005). Deregulation of the centrosome cycle and the origin of chromosomal instability in cancer. *Adv Exp Med Biol* **570**, 393–421.

[27] Shreeram S, Hee WK, and Bulavin DV (2008). Cdc25A serine 123 phosphorylation couples centrosome duplication with DNA replication and regulates tumorigenesis. *Mol Cell Biol* **28**, 7442–7450.

[28] Ng HK, Lau KM, Tse JY, Lo KW, Wong JH, Poon WS, and Huang DP (1995). Combined molecular genetic studies of chromosome 22q and the neurofibromatosis type 2 gene in central nervous system tumors. *Neurosurgery* **37**, 764–773.

[29] Vahteristo P, Eerola H, Tamminen A, Blomqvist C, and Nevanlinna H (2001). A probability model for predicting *BRCA1* and *BRCA2* mutations in breast and breast-ovarian cancer families. *Br J Cancer* **84**, 704–708.

[30] Stracker TH, Couto SS, Cordon-Cardo C, Matos T, and Petrini JH (2008). Chk2 suppresses the oncogenic potential of DNA replication-associated DNA damage. *Mol Cell* **31**, 21–32.

[31] Fukasawa K (2008). P53, cyclin-dependent kinase and abnormal amplification of centrosomes. *Biochim Biophys Acta* **1786**, 15–23.

[32] Stolz A, Ertych N, Kienitz A, Vogel C, Schneider V, Fritz B, Jacob R, Dittmar G, Weichert W, Petersen I, et al. (2010). The CHK2-BRCA1 tumour suppressor pathway ensures chromosomal stability in human somatic cells. *Nat Cell Biol* **12**, 492–499.

[33] Ganem NJ, Godinho SA, and Pellman D (2009). A mechanism linking extra centrosomes to chromosomal instability. *Nature* **460**, 278–282.

[34] Carvalho LH, Smirnov I, Baia GS, Modrusan Z, Smith JS, Jun P, Costello JF, McDermott MW, Vandenberg SR, and Lal A (2007). Molecular signatures define two main classes of meningiomas. *Mol Cancer* **6**, 64.