Inhibition of JNK2 Disrupts Anaphase and Produces Aneuploidy in Mammalian Cells*

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The JNK family members JNK1 and JNK2 regulate tumor growth and are essential for transformation by oncogenes such as constitutively activated Ras. The mechanisms downstream of JNK that regulate cell cycle progression and transformation are unclear. Here we show that inhibition of JNK2, but not JNK1, with either a dominant-negative mutant, a pharmacological inhibitor, or RNA interference caused an accumulation of mammalian cells with 4N DNA content. When observed by immunofluorescence, these cells progressed to metaphase without apparent defects in spindle formation or chromosome alignment to the metaphase plate, suggesting that the 4N accumulation is a result of postmetaphase defects. Consistent with this prediction, when JNK activity was suppressed, we observed defects in central spindle formation and chromosome segregation during anaphase. In contrast, cyclin-dependent kinase 1 activity, cyclin B1 protein, and Polo-like kinase 1 protein turnover remained intact when JNK was inhibited. In addition, continued inhibition of JNK activity did not block reentry into subsequent cell cycles but instead resulted in polyploidy. This evidence suggests that JNK2 functions in maintaining the genomic stability of mammalian cells by signaling that is independent of cyclin-dependent kinase 1/cyclin B1 down-regulation.

JNK1 and JNK2 are members of the mitogen-activated protein kinase family, which also includes the prototypical family members extracellular signal-regulated kinase and p38. Mitogen-activated protein kinases are components of signal transduction pathways that connect extracellular stimuli to intracellular responses such as modulation of cell viability, cell cycle regulation, and gene expression (1–3). JNK1 and JNK2 are ubiquitously expressed and are generally considered to share redundant functions in apoptosis and transformation and differential functions in T cell differentiation (3–6). However, recent studies have shown that the stress-induced apoptotic function previously attributed to both isoforms is actually a function specific to JNK1 (7, 8). The function of JNK2, therefore, has become less clear. This suggests that JNK functions in transformation may also be isoform-specific and should be addressed in a way that differentiates between the contributions of JNK1 and JNK2.

Ras-activating mutations have been identified in close to 35% of human cancers (9). Transformation by Ras requires JNK activity, which has made JNK an attractive target for cancer therapy (10–12). Targeting JNK for cancer therapy is also supported by studies showing that JNK activity is elevated in human tumors and that loss of JNK function inhibits tumor growth in mice (13–16). The transforming mechanism downstream of JNK is not entirely clear, but it may work through the phosphorylation of c-Jun, which in turn regulates transcription of cell cycle regulators. In addition to this pathway, JNK may have a more direct function in cell cycle regulation. We have shown that JNK localizes to centrosomes and is active in this compartment from early S phase through late anaphase with peak activity at metaphase (17). While total soluble JNK activity also increases during mitosis (18, 19), neither JNK1 nor JNK2 has been shown to function in mitosis.

To address whether JNK1 or JNK2 functions in mitosis, we used three loss of function approaches: blocking JNK activation by ectopic overexpression of a JNK dominant-negative mutant, inhibiting JNK activity with the specific pharmacological inhibitor SP600125, and down-regulating JNK isoform expression with RNA interference. The mammalian cell lines used in this study include human cervical carcinoma (HeLa) cells, human small lung carcinoma (Calu-1), and Chinese hamster ovary (CHO) cells. All three methods produced an accumulation of cells with 4N DNA content. The cells with 4N DNA content were shown to be multinucleate cells with decondensed DNA rather than cells blocked in a particular stage of mitosis. Examination of the morphology of cells progressing through mitosis in the presence of JNK inhibitor suggests that polyploidy results from defects in chromosome segregation and central spindle formation during anaphase B. RNA interference showed that the loss of the JNK2 isoform, but not the JNK1 isoform, produced an accumulation of 4N cells. At later time points, apoptotic or polyploid populations were elevated depending on cell type, suggesting that whether multinucleate cells would be eliminated or reenter subsequent cell cycles was dependent on cellular context. We also found that while inhibition of JNK delayed mitotic progression, it did not delay cyclin B1 degradation or down-regulation of cyclin-dependent kinase 1 (Cdk1) activity. This suggests that JNK may regulate anaphase progression through a mechanism further downstream of or independent of Cdk1/cyclin B1.

EXPERIMENTAL PROCEDURES

Cell Culture—Derivation of Calu-1 7-5, 8-5, and 8-30 clones has been described previously (20). Additional clones were derived by limiting...
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Dilution of Calu-1 cells transiently transfected with FLAG-JNK1(T183A,Y185F) dominant-negative mutant, abbreviated as JNK(AFP), and selected in 0.5 mg/ml G418 for 2 weeks. CHO, human cervical carcinoma (HeLa), human small lung carcinoma (Calu-1), and human embryonic kidney 293 cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 10 mM HEPES (pH 7.4), penicillin (100 units/ml), and streptomycin (100 μg/ml).

Synchronization—For the metaphase block, CHO cells were plated at 70% confluency on acid-etched poly-L-lysine coated coverslips and allowed to attach for 4 h. Attached cells were synchronized by blocking in early S phase with 2.5 mM thymidine (Sigma) for 12 h, releasing in fresh media for 5 h, and then blocking in mitosis with 0.05 μM nocodazole (Sigma) for 5 h. Synchronized cells were gently washed with fresh media, returned to 37 °C, and fixed at 0Min via a 5-min interval with 200 mM K-PIPES (pH 7.6), 5 mM EGTA, 2 mM MgCl2 at room temperature. Cells were then permeabilized in 0.5% Triton X-100 in PBM for 20 min and washed with TBST (50 mM Tris (pH 7.6), 150 mM NaCl, 0.1% Tween 20). Coverslips were then incubated for 1 h at 37 °C with primary antibodies diluted in TBST, washed in TBST, and incubated for 1 h at 37 °C with secondary antibodies diluted 1:400 in TBST. After washing in TBST, coverslips were counterstained with 0.1% DAPI (transfection following plasmid transfection, Invitrogen) according to the manufacturer’s instructions. Cells were transfected with siRNA using Oligofectamine (Invitrogen) or LipofectAMINE (transfection following plasmid transfection, Invitrogen) according to the manufacturer’s protocol. For Western blots showing reduction of endogenous protein, the caspase inhibitor Z-VAD (Sigma) was included at a concentration of 100 μM in all cultures post-transfection.

Cdkt1 Kinase Assay—Cells were lysed in lysis buffer (20 mM HEPES (pH 7.4), 150 mM NaCl, 2 mM ethylene glycol bis-β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 50 mM glycerophosphate, 1% Triton X-100, 1 mM dithiothreitol, 2 mM guanidine, 5 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride) for 10 min on ice and cleared by centrifugation. Samples were then incubated with anti-Cdk1 monoclonal antibody (Santa Cruz Biotechnology) for 1 h at 4 °C, and then protein A–agarose beads were added for an additional 30 min. Immunoprecipitates were washed with lysis buffer and incubated with 10 μg of histone H1 (Sigma), 15 μM ATP, 10 μM of [γ-32P]ATP in kinase buffer (25 mM HEPES (pH 7.6), 25 mM β-glycerophosphate, 25 mM MgCl2, 2 mM dithiothreitol) for 30 min at 30 °C. Reactions were stopped by addition of SDS sample buffer and heating to 100 °C for 5 min. Sample proteins were separated by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography.

RESULTS

To determine whether JNK activity is important for cell cycle progression, human small lung carcinoma (Calu-1), human cervical carcinoma (HeLa), and CHO cells were treated with SP600125, a pharmacological inhibitor of JNK (21). We focused on phenotypes that were common to all three cell lines and independent of differences in cellular background. The minimal effective dose of SP600125 was first determined in CHO cells to be 15 μM, which inhibited 80% of JNK activity (Fig. 1, A and B). In contrast, SP600125 showed no inhibition of the related kinase JNK2 below 15 μM and only 2% inhibition of the related kinase JNK3 below 15 μM (data not shown). The IC50 for inhibition of JNK2 in CHO cell culture was 8.5 μM with 25 μM providing 70% inhibition of JNK2 and only 2% inhibition of JNK1 (Fig. 1C). Treatment of Calu-1, HeLa, and CHO cells with 15 μM SP600125 for 72 h caused a 2.0-fold increase in S phase HeLa cells but not Calu-1 or CHO cells (Table I). This accumulation of S phase cells is, therefore, dependent on cellular background. More interestingly, this treatment caused an accumulation of cells with 4N DNA content in all three cell lines. Specifically treatment of Calu-1, HeLa, and CHO cells with 15 μM SP600125 for 72 h caused 1.6-fold (p = 0.004), 3.5-fold (p = 0.009), and 3.0-fold (p = 0.000) increases in tetraploid cells, respectively (Fig. 1D and Table I). To further confirm this observation, we screened Calu-1 small lung carcinoma clones expressing a dominant-negative mutant, FLAG-human JNK(AF2), for changes in cell cycle distribution (20). FLAG-JNK(AF2) expression was detected by Western blotting (Fig. 2A). When plated at equal density and cultured for 18 h, JNK(AF2)-expressing cultures had increased percentages of cells with 4N DNA content (Fig. 2B).

To assess the relative contributions of JNK1 and JNK2 isoforms to the 4N population accumulation, we designed siRNA to block expression of human JNK1 and JNK2 in the human cells lines HeLa and Calu. The specificity of JNK1- and JNK2-targeting siRNAs was tested by transfecting HeLa cells with hemagglutinin epitope-tagged JNK1 or FLAG epitope-tagged JNK2 followed by transfection with JNK1 or JNK2 siRNA. After 24 h, expression of JNK1 and JNK2 was assessed by Western blotting against the epitope tags. Treatment of HeLa cells with JNK1 siRNA specifically blocked greater than 95% of JNK1 and JNK2 expression, respectively (Fig. 2C). The effects of reducing JNK expression on cell cycle progression were determined by transfecting HeLa and Calu cells with
isoform-specific siRNAs and analyzing their cell cycle profiles with propidium iodide staining and flow cytometry. Changes in cell cycle profiles were first observed at 72 h post-transfection. Down-regulation of JNK1 expression did not produce significant changes in the percentage of viable cells with 4N DNA content (Fig. 2, D and E). However, down-regulation of JNK2 produced a 2-fold accumulation of viable cells with 4N DNA content in both HeLa and Calu cells by 96 and 120 h, respectively (Fig. 2, D and E). These data show that JNK2, but not JNK1, is critical for proper mitotic progression in human cells.

The increase in the 4N cell population suggests that JNK inhibition causes a mitotic block or prolonged G2/M progression. However, the accumulation of 4N cells could also be the result of an accumulation of cells that have not undergone proper chromosome segregation during one cell cycle but have still exited mitosis and reentered G0/G1. In the latter case, subsequent rounds of the cell cycle would be expected to produce cells with greater than 4N DNA content. To test this hypothesis, polyplody was assessed in HeLa, Calu-1, and CHO cells treated with SP600125. CHO cells were treated with increasing doses of SP600125 for 72 h. Accumulation of polyploid cells was observed with as little as 7 μM SP600125 with 90% of the cells becoming polyploid in the presence of 10 μM SP600125 (Fig. 3, A and B). In contrast, neither of the p38 inhibitors SB202190 and SB203580 nor the mitogen-activated protein kinase/extracellular signal-regulated kinase inhibitor PD98059 caused polyplody (Fig. 3A). Treatment of Calu-1, HeLa, and CHO cells with 15 μM SP600125 for 72 h caused 3.5-fold (p = 0.000), 4.6-fold (p = 0.001), and 8.1-fold (p = 0.000) increases in polyploid cells, respectively (Table I). Similar results were seen with Calu clones expressing the dominant-negative JNK(APF) mutant. When the clones were assessed for DNA content by propidium iodide staining and flow cytometry, up to a 15-fold increase in polyplody was observed in high expressing clones, and up to a 12-fold increase was observed in low expressing clones (Fig. 3, C–F). No significant increases in polyplody were detected in JNK siRNA-treated cultures by propidium iodide staining and flow cytometry analysis (Fig. 2F). One possible reason that we did not observe polyplody with the JNK siRNA is that the efficiency of
JNK inhibition using siRNA may be below the threshold for detecting the polyploid phenotype. Alternatively, the slower nature of this inhibition method, which requires degradation of pre-existing protein, could suggest that the polyploid cells do not survive in culture long enough to detect an accumulation. In support of this, JNK2 siRNA caused a significantly increased sub-G0 population, which is indicative of DNA fragmentation, a hallmark of apoptosis (Fig. 3G). Thus, it is possible that over longer time periods the fate of polyploid cells that result from specific JNK2 inhibition is apoptosis. This function of JNK2 in protecting cells from apoptosis contrasts with the known function of JNK1 in promoting apoptosis. The pharmacological JNK inhibitor SP600125 and the JNK(APF) dominant-negative mutant inhibit both the JNK1 and JNK2 isoforms. Thus, if the polyploid cells would have normally undergone apoptosis in a JNK1-dependent manner, SP600125 and the dominant-negative mutant would have blocked this process. This could also explain why the loss of polyploid cells and apoptosis are only detected when JNK2 function is inhibited without inhibiting JNK1.

To determine which stage of mitosis was defective, cells were treated with carrier, 20 μM SP600125, 20 μM SB202190, or 20 μM SB203580 for 72 h and their mitotic morphology was assessed by DAPI DNA staining and microscopy. CHO cells treated with SP600125, did not appear to be blocked in any stage of mitosis. However, the treated cells had enlarged and multilobed nuclei, which were not seen in cells treated with carrier or the p38 inhibitors (Fig. 4A). SP600125 also produced multinuclei in HeLa and Calu-1 cells. Similar enlarged and multilobed nuclei were detected in the Calu clones expressing the JNK dominant-negative mutant (Fig. 4B).

To determine whether this was a specific effect of the JNK2 isoform, HeLa and Calu cells were transfected with JNK1 or JNK2 siRNA for 96 or 120 h, respectively, stained with propidium iodide, and analyzed by flow cytometry for DNA content. 4N peaks are marked. Quantification of flow cytometry with the mean ± S.D. for three data sets shows that JNK2 but not JNK1 siRNA causes 4N accumulation.

In inhibition of JNK produces tetraploid cell accumulation. A, stable Calu cell lines express FLAG-tagged JNK(T183A,Y185F) (also called FLAG–JNK(APF)) dominant-negative mutant. Whole cell lysates from Calu clones stably transfected with vector (7-5) or FLAG–JNK(APF) (8-5 and 8-30) were Western blotted with an anti-FLAG antibody. B, dominant-negative cells lines have increased percentages of cells with 4N DNA content. Calu clones were plated, incubated for 18 h, and analyzed by propidium iodide staining and flow cytometry for DNA content. C, HeLa cells were transiently transfected with vector (–), hemagglutinin-tagged JNK1 (HA-JNK1), or FLAG-tagged JNK2, incubated for 4 h, and then transfected with JNK1 or JNK2 siRNA. After an additional 20 h, anti-actin, anti-hemagglutinin, and anti-FLAG Western blotting was performed. D, HeLa cells were transfected with fluorocytometry and analysis for DNA content. 4N peaks are marked. Quantification of flow cytometry with the mean ± S.D. for three data sets shows that JNK2 but not JNK1 siRNA causes 4N accumulation.
the microtubules and DNA, respectively. As expected, JNK2, but not JNK1, siRNA caused a 4-fold accumulation of HeLa cells with enlarged and multilobed nuclei (Fig. 4, C and E). This indicates that JNK2, but not JNK1, is required for proper chromosome segregation and cytokinesis in HeLa cells. These RNA interference-induced multinuclei, present after one cell cycle (HeLa and Calu-1) with reduced JNK expression, were more defined and less globular than the multinuclei seen with the long term pharmacological JNK inhibition (HeLa, Calu-1, and CHO) and the chronic suppression of JNK activity in the dominant-negative JNK-expressing Calu-derived clones (Fig. 4, A and B). However, all three methods produced cells with enlarged nuclei, showing that chromosome segregation and cytokinesis during mitosis did not occur when JNK was inhibited. Interestingly neither JNK1 nor JNK2 siRNA significantly altered the overall percentage of cells morphologically progressing through mitosis as assessed by microscopy (Fig. 4D). Therefore, because these cells were not blocked in a specific stage of mitosis, the nature of the defect leading to multinucleation was not clear. Although the inhibition of JNK appears to affect S phase progression in HeLa cells, CHO and Calu-1 cells progressed normally through S phase in the presence of JNK inhibitor (Table I). This suggests that the defect leading to multinucleation, which was common to all three cell lines, was occurring after S phase. To determine whether cells were properly progressing through G2 and mitosis when JNK was inhibited, analysis of cellular morphology by immunofluorescence was performed.

Several possible mechanisms may account for the multinucleation observed in cells depleted of JNK activity, including defects in chromosome condensation during G2, chromosome alignment to the metaphase plate, spindle formation, chromosome segregation, and cytokinesis. To determine whether JNK inhibition caused defects in global microtubule nucleation and/or growth, CHO cells were treated with nocodazole to depolymerize existing cytoskeletal microtubules. Nocodazole was then washed from the cell culture, and microtubules were allowed to nucleate and regrow at 37 °C in fresh cell culture media. Microtubule nucleation and regrowth were intact in the presence of 25 μM SP600125, suggesting that JNK is not required for these processes (Fig. 5A). More importantly, morphologically normal bipolar spindles with chromosomes...
aligned at the metaphase plate are present in HeLa, Calu-1, and CHO cells treated for 24 h with 15 μM SP600125. This indicates that in mammalian cells, chromosome condensation, chromosome alignment to the metaphase plate, and spindle formation do not require JNK activity (Fig. 5B and data not shown).

To determine whether defects occurred after metaphase during chromosome segregation of anaphase A and B, CHO and...
HeLa cells were first synchronized in metaphase with a single thymidine block followed by a nocodazole block. Synchronized cells were then released into fresh culture media, followed through anaphase in the presence or absence of JNK inhibitor by fixation every 15 min, and visualized by immunofluorescence imaging. In the absence of SP600125, central spindle formation, midbody formation, and cytokinesis were detected at 30, 45, and 60 min, respectively (Fig. 6A and data not shown). In the presence of SP600125, sister chromatid separation was initiated during anaphase A. However, central spindle formation during anaphase B, chromosome segregation, and cytokinesis were defective (Fig. 6, A and B, and data not shown). SP600125 produced chromosome segregation defects in over 90% of anaphase cells (Fig. 6C). In contrast, an inhibitor of the related mitogen-activated protein kinase p38 did not cause chromosome segregation defects (Fig. 6C). These results suggest that while JNK activity is dispensable for metaphase spindle formation, JNK activity is important for central spindle formation for cells to complete chromosome segregation during anaphase B and undergo proper cytokinesis.

Progression through anaphase and mitotic exit is regulated in part by a complex network of kinases that is just beginning to be understood in mammalian systems. One key central event is the destruction of cyclin B1 and the down-regulation of the kinase activity of its associated partner, Cdk1 (see Fig. 8A). The cyclin B1 degradation and the Cdk1 inactivation is initiated at the metaphase to anaphase transition and when complete allows mitotic exit (22). How cyclin B1/Cdk1 controls anaphase B and mitotic exit is still unclear. However, several downstream pathways have been implicated in this process, including the Plk1 pathway (23). Plk1 is also regulated by degradation during mitotic exit in human cells (24). To determine whether JNK activity is required for the down-regulation of Cdk1 activity, cyclin B1 protein degradation, and/or Plk1 protein degradation, HeLa cells were synchronized by double thymidine block, treated with JNK inhibitor just prior to entry into metaphase, and then assayed for Cdk1 kinase activity, cyclin B1 protein levels, and Plk1 protein levels as they progressed through mitosis. Mitotic exit was delayed by about 4 h in the presence of 15 μM SP600125 (Fig. 7A). In carrier-treated cells, the majority of 4N cells exited mitosis and entered G1 by 9 h postrelease from thymidine block (Fig. 7B). However, in the presence of SP600125, most cells were still in the 4N state by 9 h (Fig. 7B). Duplicate samples from this synchronization were assayed for Cdk1 kinase activity. While SP600125 increased the duration of the 4N state, it did not prolong Cdk1 kinase activity and instead showed a reduction in overall Cdk1 kinase activity (Fig. 7C). This shows that JNK is not prolonging mitotic cell turnover by inhibiting the Cdk1 down-regulation required for mitotic exit. Cyclin B and Plk1 protein down-regulation was also assessed in these samples by Western
blotting (Fig. 7D). Neither cyclin B1 nor Plk1 down-regulation was blocked by SP600125 (Fig. 7D). This suggests that JNK acts further downstream than the Cdk1/cyclin B1 pathway through a mechanism independent of Plk1 protein degradation.

**DISCUSSION**

In this study, we identified a new function for JNK in cell cycle control. We showed that while JNK activity was dispensable for chromosome condensation, chromosome alignment to the metaphase plate, and spindle formation, loss of JNK function interfered with chromosome segregation and central spindle formation in mammalian cell lines. In three different cell lines (HeLa, Calu-1, and CHO) the loss of JNK activity also led to an accumulation of polyploid cells, indicating that there is a breakdown in genomic segregation and either a direct or an indirect block in cytokinesis. Accumulation of polyploid cells after total JNK inhibition has also been detected in breast cancer cells (25). JNK1 and JNK2 are ubiquitously expressed in mammalian cells and have long been thought to have redundant functions. However, we have found that inhibition of specific JNK1 or JNK2 isoforms produces different effects on the mammalian cell cycle. Down-regulation of JNK1 did not affect cell cycle progression or viability of either human cell line (HeLa or Calu-1). In contrast, inhibition of JNK2 produced an accumulation of cells with 4N DNA content in both cell lines, showing that this function is specific to the JNK2 isoform. It is interesting to note that while down-regulation of JNK2 also caused an accumulation of polyploid Calu-1 cells, it did not produce an accumulation of polyploid HeLa cells. Instead down-regulation of JNK2 appeared to cause substantial apoptosis in HeLa cells, which may be the final fate of cells with mitotic defects in this cellular background when JNK1 activity remains intact. JNK1 has a well established role in stress-induced apoptosis, and it is interesting to speculate that it may also be involved in apoptosis induced by mitotic defects. These functions contrast with those of the related mitogen-activated protein kinases extracellular signal-regulated kinase and p38 in spindle microtubule attachment to kinetochores and in the spindle assembly checkpoint, respectively (26, 27).

Previous studies have suggested that JNK functions in either S phase or G2 phase of the mammalian cell cycle (20, 25). In contrast, we found that JNK function in S phase is cell line-specific. Inhibition of JNK resulted in S phase delay in only one of three cell lines tested. However, we found that JNK produced mitotic defects in all three cell lines tested. We followed cells through mitosis to determine where the defect occurred and found that while cells progressed through G2 to metaphase without apparent defects, chromosome segregation during anaphase was disrupted. As previously mentioned, JNK2 does not appear to be required for passing the spindle assembly checkpoint or for initiation of sister chromatid separation during anaphase A. Sister chromatid separation appears to be initiated when JNK is inhibited, although whether it is completed is still under investigation. In either case, central spindle formation, poleward movement of the chromatids dur-
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Fig. 8. JNK1 promotes apoptosis, while JNK2 regulates mitotic progression through mechanisms downstream of Cdk1/cyclin B1. A, JNK2 is not required for the down-regulation of Cdk1 kinase activity or cyclin B1 protein degradation. This suggests that JNK2 may function in pathways further downstream of the anaphase-promoting complex (APC). JNK2 may function by affecting the activity or localization of chromosomal passenger proteins such as Plk1 or other proteins necessary for anaphase progression. B, JNK1 has a well-established role in promoting apoptosis induced by intracellular and extracellular stimuli. JNK2, however, plays a contrasting role in promoting cell viability. The JNK isoforms are further functionally distinguished from each other by the newly found function for JNK2 in mitotic progression, a function that is not shared by JNK1.

Several alternative mechanisms that contribute to the regulation of anaphase B and cytokinesis may be regulated by JNK. In mammalian cells, actin depolymerization is often accompanied by failure of central spindle formation, implicating actin polymerization in the initiation of anaphase B (29). Interestingly JNK is required for proper actin dynamics in Drosophila embryos and therefore may contribute to central spindle formation through the regulation of actin dynamics (30). Proper regulation of actin dynamics is also necessary for cytokinesis. It is known that degradation of cyclin B during anaphase activates a Rho GTPase, called Pebble in Drosophila, which is required for ring assembly and cytokinesis (31–33). If JNK regulates cytokinesis through the regulation of actin dynamics, this study shows that it is most likely acting independently of this Rho/Cyclin B1/Cdk1 pathway. Further study is necessary to determine whether JNK is acting downstream of this Rho pathway to regulate actin dynamics during central spindle formation and/or cytokinesis.

Once formed, elongation of the anaphase B central spindle is regulated by opposing forces of the kinesin and dynein families of motor proteins on central spindle microtubules (34). Kinesins regulate the sliding of central spindle microtubules to push spindle poles apart, while dyneins regulate astral microtubule dynamics to pull spindle poles apart. JNK interacts with the Kif3 kinesin on cytoplasmic microtubules (35), raising the possibility that JNK may also interact with mitotic kinesin family members. In addition, JNK associates with the JNK-interacting protein family of scaffolding proteins, which in turn associate with kinesins (36, 37). Yet another possible link between JNK and dynein regulation is the kinase Fyn. Fyn interacts with dynein and is an upstream regulator of the JNK pathway (38–41). It is therefore possible that interactions between JNK and kinesin motor proteins or participation of JNK in a dynein regulatory pathway with Fyn may function to ensure the completion of the mitosis and the maintenance of genomic stability.

This new function for JNK in the regulation of mitosis is a possible mechanism for how JNK acts in transformation and tumor progression signaling pathways. Loss of JNK2 function may result in an inhibition of cell growth by blocking chromosome segregation and cytokinesis. If the loss of JNK2 function leads to slowed cell cycle progression in nontransformed contexts, as it does in the transformed cells line models presented here, then loss of JNK2 may be a useful target for treating hyperproliferative disorders such as cancer. This would suggest that the loss of JNK2 function would have little effect on tumor incidence and instead would reduce tumor progression. This model is supported by the result that JNK2-null mice are resistant to mitogen-induced tumor growth and malignant transformation (16). This is further supported by a recent finding that JNK2 is constitutively activated in primary glial tumors (42).

The presence of MKK4-inactivating mutations (MKK4 is a kinase directly upstream of JNK activation) in some human tumors (43) is inconsistent with this model. However, MKK4 mutation-associated tumorigenesis would most likely be related to the loss of JNK1 functions and therefore the loss of DNA damage-induced apoptosis pathways (Fig. 8B). Reduced apoptosis of cells with DNA damage would lead to an accumulation of mutations in cells, thus sensitizing organisms to tumorigenesis. This model is supported by a report that JNK1-null mice are sensitized to mitogen-induced tumor incidence (44).

Several JNK inhibitors are being developed and tested in clinical trials for the treatment of neurological diseases, metabolic disorders, inflammatory conditions, and cancer (11). However, these inhibitors are not specific for JNK isoforms. Our studies would suggest that this could reduce the efficacy of these inhibitors and increase unwanted side effects in therapeutic applications. The data presented here show that JNK2 participates in the essential process of chromosome segregation during anaphase in mammalian cells. In a transformation background, the down-regulation of JNK2 would be expected to reduce tumor cell growth. In addition, one would predict that the genetic loss of JNK2 function would create a tumorigenesis-
resistant phenotype. In contrast, inhibition of JNK1 would be predicted to lead to an increased frequency of survival of cells with potentially oncogenic mutations, thereby sensitizing organisms to tumorigenesis. It is, therefore, essential to consider the distinct functions of JNK1 and JNK2 in differentiation, apoptosis, and proliferation, specific targeting of JNK1 or JNK2 may produce more precise and effective therapies by reducing harmful or unwanted side effects that result from inhibiting a cellular pathway unrelated to disease progression.

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