Checkpoint kinase 1–induced phosphorylation of O-linked β-N-acetylglucosamine transferase regulates the intermediate filament network during cytokinesis

Received for publication, August 10, 2017, and in revised form, September 27, 2017   Published, Papers in Press, October 11, 2017, DOI 10.1074/jbc.M117.811646

Zhe Li (李喆‡), Xueyan Li (李雪燕‡), Shanshan Nai (能姗姗‡), Qizhi Geng (耿奇志‡), Ji Liao (廖蓟‡), Xingzhi Xu (许兴智‡§), and Jing Li (李静)‡‡

From the ‡Beijing Key Laboratory of DNA Damage Response and College of Life Sciences, Capital Normal University, Beijing 100048, China and the §Guangdong Key Laboratory of Genome Stability & Disease Prevention, Shenzhen University School of Medicine, Shenzhen, Guangdong 518060, China

Edited by Gerald W. Hart

Checkpoint kinase 1 (Chk1) is a kinase instrumental for orchestrating DNA replication, DNA damage checkpoints, the spindle assembly checkpoint, and cytokinesis. Despite Chk1’s pivotal role in multiple cellular processes, many of its substrates remain elusive. Here, we identified O-linked β-N-acetylglucosamine (O-GlcNAc)-transferase (OGT) as one of Chk1’s substrates. We found that Chk1 interacts with and phosphorylates OGT at Ser-20, which not only stabilizes OGT, but also is required for cytokinesis. Phospho-specific antibodies of OGT–pSer-20 exhibited specific signals at the midbody of the cell, consistent with midbody localization of OGT as reported previously. Moreover, phospho-deficient OGT (S20A) cells attenuated cellular O-GlcNAcylation levels and also reduced phosphorylation of Ser-71 in the cytoskeletal protein vimentin, a modification critical for severing vimentin filament during cytokinesis. Consequently, elongated vimentin bridges were observed in cells depleted of OGT via an siOGT-based approach. Lastly, expression of plasmids resistant to siOGT efficiently rescued the vimentin bridge phenotype, but the OGT–S20A rescue plasmids did not. Our results suggest a Chk1–OGT–vimentin pathway that regulates the intermediate filament network during cytokinesis.

O-Linked β-N-acetylglucosamine (O-GlcNAc) transferase (OGT) is the sole enzyme in humans that catalyzes the adding of the O-GlcNAc moiety to the Ser/Thr residues of nuclear and cytoplasmic proteins (1). The reverse reaction is catalyzed by O-GlcNAcase (OGA). The OGT/OGA pair accounts for all the adding and reversible removal of the O-GlcNAc group in human proteins. Despite the significant roles of O-GlcNAc in protein modification and signal transduction, its substrates have just started to unfold. Extensive crosstalk between O-GlcNAcylation and phosphorylation has long been documented; O-GlcNAc either vies with phosphate groups for the same or adjoining Ser/Thr sites, or in some other cases, promotes it (1, 2).

Recently, a vital role of O-GlcNAc in cytokinesis has emerged. It has been well known that cytokinesis is an intricate process fine-tuned by a plethora of kinases (3). Now through a proteomic approach, 141 proteins involved in spindle assembly and cytokinesis have been newly shown to be O-GlcNAcylated (4). OGT localizes to the midbody (4, 5), an organelle involved in cytokinesis. Moreover, over-expression of OGT induces the polyploidy phenotype, indicative of cytokinesis defects (4). Furthermore, OGT has also been shown to modify vimentin (5), one of the intermediate filament proteins expressed in mesenchymal, cultured, and tumor cells (6).

Phosphorylation of vimentin is regulated at multiple levels during the cell cycle. Vimentin is phosphorylated at G2/M by cyclin-dependent kinase 1 (CDK1) at Ser-55 (7), which primes vimentin for subsequent phosphorylation by polo-like kinase 1 (PLK1) at Ser-82 (8), and consequent inhibition of vimentin filament formation. Besides, vimentin is also phosphorylated by Aurora B at Ser-72 and Rho kinase at Ser-71, leading to localization of phosphorylated vimentin to the cleavage furrow from anaphase to cytokinesis (9–11). pSer-71 inhibits vimentin filament formation (10), and it is enhanced upon either OGT or OGA over-expression (5). When 12 mitosis-related phosphorylation sites were mutated to Ala and the resultant VIMS/A-SA knock-in mice were generated, the mice manifested binucleation and aneuploidy in the lens, age-related cataracts, and skin defects, suggestive of premature aging (12, 13).

Checkpoint kinase 1 (Chk1) is an essential kinase that maintains genome integrity. Chk1 orchestrates replication, DNA damage checkpoints, the spindle assembly checkpoint, and cytokinesis (14, 15). Abrogation of Chk1 induces cytokinetic regression, binucleation, and myriad cytokinetic defects (14).
Chk1 phosphorylates OGT in cytokinesis

In a previous investigation (16), OGT was identified in a phospho-proteomic screen of Chk1, and Ser-20 of OGT was identified as one of the phosphorylation sites. We constructed OGT(S20A) plasmids accordingly, and verified this possibility by in vitro kinase (IVK) assays. Commercially available GST-Chk1 was incubated with GST-OGT or GST-OGT(S20A) proteins and subject to IVK assays. Chk1 phosphorylated OGT efficiently but not the OGT(S20A) mutant (Fig. 2A). The amino acid residues surrounding Ser-20 were examined for a Chk1 kinase consensus motif, and a minimum consensus for Chk1-dependent phosphorylation was identified (Fig. 2B).

We then prepared a phospho-specific antibody toward OGT—pSer-20 as described in “Experimental Procedures” and adopted IVK assays with the pSer-20 antibodies. Chk1 efficiently phosphorylated wild-type (WT) OGT, but not the OGT(S20A) mutant (Fig. 2C). We also tested whether OGT is phosphorylated by Chk1 in vivo. The pSer-20 antibodies specifically detected a band in the HeLa cell extracts, which was significantly attenuated when cells were treated with UCN-01, a specific inhibitor of Chk1 (Fig. 2D). Then HeLa cells were transfected with vectors or HA-OGT-WT plasmids, and synchronized in cytokinesis or left unsynchronized. When treated with UCN-01, the signal of the antibody markedly decreased (Fig. 2E). We sought to determine when the phosphorylation event occurs during the cell cycle. HeLa cell extracts were synchronized by thymidine-nocodazole (Noc) block, and then released. Cell extracts were taken at different time points, prepared, and blotted with the pSer-20 antibodies and subject to flow cytometry analysis. A crisp band was discernable only after the percentage of mitotic cells started to decline (Fig. 2F, lane 2). These results suggest that Chk1 specifically phosphorylates OGT at Ser-20.

Chk1-dependent phosphorylation of pSer-20 stabilizes OGT

OGT has been shown to be poly-ubiquitinated and subject to proteasome-mediated degradation (17). Therefore, we examined whether Chk1-dependent phosphorylation of OGT at Ser-20 modulates OGT abundance. HeLa cells were transfected with HA-OGT WT or S20A plasmids, and treated with cycloheximide (CHX), a protein synthesis inhibitor. Cell extracts were collected at different time points, and subject to immunoblotting (IB). OGT(S20A) displayed significantly shorter half-life than WT (Fig. 3, A and B). Moreover, we examined the ubiquitination levels of OGT by transfecting HA-Ub and Myc-OGT WT or S20A plasmids, and synchronized in cytokinesis or left unsynchronized. When treated with UCN-01, we observed elevated ubiquitination levels in OGT(S20A) (Fig. 3C), consistent with more rapid turnover of OGT(S20A). In addition, we examined interplay between Chk1 kinase activity and OGT ubiquitination levels by treating the cells with UCN-01. As shown in

The detailed mechanism of Chk1 in cytokinesis remains unresolved. In an effort to identify more potential Chk1 interacting proteins, we performed mass spectrometry (MS) analysis following immunoprecipitation (IP) and found OGT. We show here that Chk1 interacts with and phosphorylates OGT at Ser-20, which is a prerequisite of OGT stability. Attenuation of OGT protein levels by siRNA treatment or the S20A mutation hampers the phosphorylation of vimentin at Ser-71, resulting in a failure of vimentin bridge disassembly. Our results not only exemplified another crosstalk between Chk1 and cytokinesis, but also revealed the molecular underpinning of Chk1’s role in cytokinesis.

Results

Chk1 associates with OGT

To investigate potential proteins that interact with Chk1, we performed an IP experiment using anti-Chk1 antibodies followed by MS analysis and identified OGT (Fig. 1A). To validate the interaction, HeLa cells were subject to IP experiments using anti-Chk1 and anti-OGT antibodies. OGT was detected in anti-Chk1 immunoprecipitates (Fig. 1B), suggesting that endogenous Chk1 and OGT co-immunoprecipitate. To exclude the possibility that the endogenous interaction was because of nonspecificity of the antibodies, we transfected HeLa cells with epitope-tagged Chk1 and OGT plasmids and performed co-IP experiments. As shown in Fig. 1, C and D, both HA-tagged and Myc-tagged OGT interacted with FLAG-Chk1. To test whether the interaction between OGT and Chk1 was direct, we transfected HA-OGT into HeLa cells and used recombinant GST-Chk1 to perform pulldown assays (Fig. 1E). GST-Chk1 could pull down HA-OGT (Fig. 1E). Reciprocally, GST-OGT also could pull down FLAG-Chk1 (Fig. 1F). Taken together, these data suggest that Chk1 interacts with OGT.

Chk1 phosphorylates OGT at Ser-20

Figure 1. Chk1 associates with OGT. A, Chk1 was immunoprecipitated from HeLa cells, and samples were analyzed by SDS-PAGE followed by Coomassie Blue staining. Proteins interacting with Chk1 were identified by mass spectrometry. IgG immunoprecipitates were used as a negative control. Numbers indicate molecular weight markers (in kDa). B, co-IP between endogenous Chk1 and OGT. Chk1 immunoprecipitates were blotted with anti-Chk1 and anti-OGT antibodies. OGT was detected in anti-Chk1 blotted with anti-OOGT antibodies. C and D, co-IP between exogenous Chk1 and OGT. HeLa cells were transfected with FLAG-Chk1, HA-OGT (C) or Myc-OGT (D), and subjected to IP and IB experiments using the antibodies indicated. E and F, pulldown experiments using GST-Chk1 (E) or GST-OGT (F) to pull down transfected HA-OGT (E) or FLAG-Chk1 (F).
Chk1 phosphorylates OGT in cytokinesis

Fig. 3D, OGT displayed more robust poly-ubiquitination upon UCN-01 treatment. Taken together, these results suggest that Chk1 plays a pivotal role in stabilizing OGT.

OGT–pSer-20 localizes to the midbody

We then addressed the question concerning in which intracellular compartments the phosphorylated OGT resides. As shown in Fig. 4A, pSer-20 of OGT localizes to the midbody, suggesting that phosphorylated OGT could take part in cytokinesis. This is also in line with previous findings that OGT localizes to the midbody (5). To determine that the localization was not because of fortuitous binding of the phospho-antibody, we adopted two siRNA oligos targeting OGT, both of which efficiently depleted OGT protein levels (Fig. 4B). Upon siOGT, the midbody localization of pSer-20 significantly decreased (Fig. 4C, D and E). Upon siOGT, the midbody localization pattern was dependent on Chk1. When we used siCHK1 to deplete Chk1 (Fig. 4F), the midbody localization of pSer-20 was also attenuated (Fig. 4G). Upon quantitation, the midbody staining was compromised upon siCHK1 (Fig. 4H), suggesting that Chk1-dependent phosphorylation of OGT localizes to the midbody.

OGT(S20A) attenuates vimentin O-GlcNAcylation and phosphorylation

Previous investigations have implicated interplay of O-GlcNAcylation with phosphorylation in regulating vimentin (5). Specifically, both OGT and OGA over-expression boosted vimentin–pSer-71 in mitosis (5). We sought to examine whether OGT depletion or OGT(S20A) was also implicated in regulating vimentin phosphorylation during cytokinesis.

HeLa cells were transfected with two distinct oligos targeting OGT, and then synchronized to the cytokinetic stage or unsynchronized (Fig. 5A). The resultant cell extracts were subject to IB to examine O-GlcNAc together with vimentin pSer-71 levels. Our results indicate that decreased O-GlcNAc levels may thus lead to vimentin bridges. We verified our hypothesis by examining vimentin staining in siOGT cells (Fig. 5B). Although vimentin staining was lacking in the midbody area in control cells, vimentin formed conspicuous bridges in the siOGT cells (Fig. 5B). Upon quantitation, cells harboring cytokinetic defects increased markedly in siOGT cells (Fig. 5C).
OGT(S20A) fails to rescue cytokinesis defects and vimentin–pSer-71 levels

We then sought to examine the cellular defects of OGT(S20A). Rescue plasmids were constructed. They efficiently recovered endogenous OGT levels, but were resistant to siOGT treatment (Fig. 6A). In these cells, vimentin–pSer-71 levels were significantly lower in cells harboring S20A, but higher in S20D (Fig. 6A), suggesting that OGT(S20A) is detrimental to vimentin phosphorylation at Ser-71.

Indeed, when examined under the fluorescence microscopy, S20A cells did manifest vimentin bridges, as the cells depleted of OGT did, but not in the S20D counterparts (Fig. 6B). Upon quantitation (Fig. 6C), S20A contained more cytokinetic defective cells, suggesting that S20A causes cytokinetic defects via dampened vimentin–pSer-71 levels.

Chk1 depletion induces vimentin bridge formation during cytokinesis

If the Chk1–OGT–vimentin pathway stands true in modulating cytokinesis, then Chk1 should have a direct role in vimentin phosphorylation and polymerization. We utilized siCHK1 in synchronized cytokinetic cells and examined biochemically whether vimentin–pSer-71 was affected (Fig. 7A), and evidently there was a 30% decrease. Cytologically we applied the Chk1 inhibitor UCN-01 in cells, and vimentin bridges were abundant (Fig. 7, B and C). Taken together, the cytokinetic defects observed in Chk1-depleted cells could be attributed to, at least in part, failure of intermediate filaments to disassemble.

Discussion

OGT is the sole enzyme in humans that accounts for all the O-GlcNAcylation reactions. Recently an intricate role of OGT in cytokinesis emerged. Here we show that Chk1 interacts with OGT, and phosphorylates OGT at Ser-20 both in vitro and in vivo. OGT–pSer-20 localizes to the midbody, and underscores the stability of OGT. Vimentin, subject to both phosphorylation and O-GlcNAcylation, mediates the intermediate filament bridge formation and severing during cytokinesis. We discovered that attenuation of O-GlcNAcylation reduces vimentin–pSer-71 during cytokinesis. Because vimentin–pSer-71 is a prerequisite of vimentin filament disassembly, it is conceivable that vimentin–pSer-71 levels provide the molecular underpinning of cytokinesis defects induced by OGT or Chk1 depletion (Fig. 7D).

The modifications of OGT are far from being known. OGT has been shown to be ubiquitinated and deubiquitinated. The E3 ligase of OGT has recently been identified to be the histone demethylase LSD2 (18). LSD2 not only demethylates H3K4me1/me2, but also ubiquititates OGT and promotes its subsequent

Figure 3. Phosphorylation of Ser-20 promotes OGT stability. A, HeLa cells were transfected with HA-OGT or HA-OGT(S20A), treated with 20 μg/ml cycloheximide (CHX), and then collected at different time points. Cell extracts were blotted with anti-HA and anti–β-actin antibodies. B, kinetics of HA-OGT and HA-OGT(S20A) stability as shown in (A). The data are representative of three independent experiments. C and D, analysis of ubiquitination of OGT. C, HeLa cells were transfected with HA-ubiquitin (Ub) and Myc-OGT or Myc-OGT(S20A), D, HeLa cells were transfected with HA-Ub and Myc-OGT, treated with UCN-01. Ubiquitination of OGT was analyzed by IP and IB with indicated antibodies. Numbers indicated densitometric quantitation of Western blot bands. The data are representative of three independent experiments.

Figure 4. pSer-20 localizes to the midbody. A, HeLa cells were stained with anti–pSer-20 and anti–α-tubulin antibodies. Scale bar, 10 μm. B, HeLa cells were subject to siOGT treatments (two different oligos), and then extracts were immunoprecipitated with antibodies indicated. C, HeLa cells after siOGT treatment (two different oligos) were stained with anti-OGT, pSer-20, and α-tubulin antibodies, together with DAPI. D, quantitation of cells with positive OGT–pSer-20 signals showing mean ± S.D. from three independent experiments. More than 100 anaphase cells were counted in each experiment. * indicates significant differences from the control, as determined by two-tailed t test (p<0.0005, p<0.0006). E–G, the midbody localization of pSer-20 depends on Chk1. E, HeLa cells were subject to siChk1 treatment, and then extracts were immunoblotted with antibodies indicated. F, cells in (E) were stained with anti-OGT, pSer-20, and α-tubulin antibodies, together with DAPI. Then they were analyzed as in (C). White arrowheads in (C) and (F) indicate staining at the midbody; White arrows in (C) and (F) indicate absence of staining at the midbody. Insets in (C) and (F) exemplify enlargement of the midbody area. G, quantitation of results in (F), * indicates significant differences from the control (p = 0.0001).
proteome-dependent degradation, thus suppressing tumorigenesis (18). The deubiquitination enzyme has been identified to be the tumor suppressor BRCA1-associated protein-1 (BAP1) (17). BAP1 depletion efficiently decreased OGT levels (17). Besides ubiquitination, OGT is also phosphorylated by AMP-activated protein kinase (AMPK) at Thr-444 (19, 20). Phospho-mimic T444E changes the substrate selectivity of OGT (20), and Thr-444 phosphorylation abrogates the chromatin association of OGT and subsequent H2B Ser-112 O-GlcNAcylation (19). Our results thus unveil a second phosphorylation site of OGT that occurs specifically at the cytokinetic stage. How and why pSer-20 crosstalks with ubiquitination remains to be clarified, because the ubiquitination residues are yet to be identified.

Figure 5. OGT depletion impedes pSer-71 of vimentin. A, OGT depletion attenuated O-GlcNAcylation and pSer-71 phosphorylation of vimentin in cytokinetic cells. HeLa cells were first treated with two different siOGT oligos or left untreated, then synchronized in the cytokinetic phase as described in “Experimental Procedures.” The total lysates were subject to IB with the antibodies indicated. Numbers indicate levels of RL2 and vimentin–pSer-71, respectively. The data are representative of three independent experiments. B, HeLa cells were treated with siOGT (two independent oligos), then subject to immunostaining with anti-vimentin antibodies and DAPI. Scale bar, 10 μm. An elongated vimentin bridge was discernible in siOGT cells. C, HeLa cells treated with two different siOGT oligos were quantitated for anaphase arrest plus multinucleated cells, indicating defects in cytokinesis. At least 100 cells were counted. The results are mean ± S.D. of three independent experiments. * indicated significant differences from WT (p_lane1–2 = 0.005, p_lane1–3 = 0.002).

Figure 6. OGT-S20A fails to rescue cytokinesis defects and vimentin–pSer-71 levels. A, HeLa cells were stably transfected with HA vector, HA-OGT-res, WT, S20A, and S20D plasmids, and then treated with control siRNA or siOGT, as indicated. The lysates were synchronized in the cytokinetic stage and subject to IB using the antibodies indicated. Numbers indicate levels of vimentin–pSer-71. The data are representative of three independent experiments. B, the same cells in A were subject to immunostaining with anti-vimentin antibodies and DAPI. Scale bar, 10 μm. C, the same cells in A were quantitated for anaphase arrest plus multinucleated cells, indicating defects in cytokinesis. At least 100 cells were counted. The results are mean ± S.D. of three independent experiments. * indicates significant differences from WT (p_lane1–2 = 0.0003, p_lane1–4 = 0.002).
Perhaps it came as a bit of a surprise that O-GlcNAcylation of vimentin actually promotes phosphorylation at Ser-71. This is not unprecedented, however. Although O-GlcNAcyltransferase vies with phosphorylation in a great many cases, it may enhance phosphorylation at times. Indeed, when active phosphorylation sites were closely monitored in a study where O-GlcNAcyltransferase was elevated, 148 phosphorylation sites were increased and 280 phosphorylation sites were reduced (2). As mentioned previously, Chk1 pSer-151 decreased upon OGT depletion (21). Hence, it is not a simple relationship of competition between the two modifications.

We speculate that O-GlcNAcylation may augment Rho kinase activity, the kinase responsible for pSer-71 of vimentin. Multiple lines of genetic evidence are congruent with the idea. First, in endothelium-denuded rat aortas, OGT inhibition via PUGNAc (OGA inhibitor) stimulated contraction to phenylephrine, which was abolished by the Rho kinase inhibitor Y-27632 (22). Second, in the same aorta system, glucosamine was utilized to induce O-GlcNAcyltransferase of proteins by increasing the influx of the hexosamine biosynthesis pathway that feeds into the O-GlcNAc pathway. Glucosamine also elevated RhoA activity, which was negated by OGT inhibition (23). Lastly, in SKOV3 and 59M ovarian cells, siRNA targeting OGT suppressed the cellular migration and invasion, whereas Thiamet-G (OGA inhibitor) bolstered it. More importantly, Thiamet-G boosted RhoA activity and the phosphorylation of Rho kinase substrates, whereas siOGT dampered RhoA activity and subsequent Rho kinase substrate phosphorylation (24). Collectively, O-GlcNAcylation may fuel the RhoA/Rho kinase signaling pathway.

Besides cytokinesis, vimentin also plays various roles in various stages of cancer, including tumorigenesis, epithelial-to-mesenchymal transition and ultimately metastasis (25), in human hepatocellular carcinoma (26), lung cancer (27), and breast cancer (28), just to name a few. There has been increasing awareness that vimentin expression elevation correlates with more intensive cancer cell migration and invasion, which could be attributed to the interconnection between vimentin and actin (29), as filamentous vimentin controls actin stress fiber assembly and contractile actomyosin bundles, thus promoting metastasis. Alternatively, vimentin filaments could support extension of microtubule protrusions upon tumor cells detaching from the extracellular matrix (28), and in this way assist a successful tumor cell spread. In either case, an intact vimentin expression is indispensable, and decreased vimentin–GlcNAcylated (21). Chk1 phosphorylation profiles changed upon OGT depletion (21). Specifically, pThr-113 increased 2.2-fold whereas pSer-151 decreased 0.7-fold. Therefore, it is highly unlikely a simple friend or foe relationship exists between O-GlcNAcyltransferase and phosphorylation, as far as Chk1 is concerned.

Our results do not preclude the possibility that Chk1 is O-GlcNAcylated by OGT. Indeed, in a proteomic screen for proteins involved in DNA damage response, Chk1 was identified to be O-GlcNAcylated (21). Chk1 phosphorylation profiles changed upon OGT deletion (21). Specifically, pThr-113 increased 2.2-fold whereas pSer-151 decreased 0.7-fold. Therefore, it is highly unlikely a simple friend or foe relationship exists between O-GlcNAcyltransferase and phosphorylation, as far as Chk1 is concerned.

**Experimental procedures**

**Cell culture, antibodies, and plasmids**

HeLa and HEK293 cells were purchased from ATCC. Anti-OGT antibodies were from Santa Cruz Biotechnology (sc-32921), anti-O-GlcNAc antibodies (RL2) from Abcam, and anti-vimentin–pSer-71 antibodies from Abcam (ab115189). Antibodies against OGT phospho-Ser-20 (pSer-20–Ab) were raised in rabbits using the sequence of TKRML(pS)FQGLC and...
manufactured by Beijing B&M Biotech Co. OGT sequences were amplified by PCR and cloned into pcDNA3.0–3HA, resulting in pcDNA–3HA-OGT. Myc-OGT and GST–OGT plasmids were described previously (30), and were gifts from Drs. Huadong Pei and Xiaochun Yu. OGT(S20A) mutants were generated using specific primers (sequences available upon request) following the manufacturer’s instructions (QuikChange II, Stratagene).

**Cell synchronization**

Protocols to synchronize cells in the cytokinetic phase were described before (31). Briefly, cell cultures were first blocked by double thymidine, and collected 9 h after releasing from the second thymidine block.

**Transfections**

HeLa cells were transfected twice with a 24-h interval using Oligofectamine (Invitrogen) according to the manufacturer’s instructions. Transfectants were used for further experiments 24 h after the second transfection. All small interfering RNA (siRNA) oligonucleotide duplexes were purchased from Dharmacon. The control siRNA oligonucleotide duplex was CONTROLsi, CGUACGCUGGAUACUUCGAdTdT; siOGT 1#, GCAGUAGCUUGGAGAUAUc dTdT; siOGT 3#, GCACGG CUCUGAACCUCUAA dTdT; siCHK1, CGTATTGTGAACAA GATGTGTGATTCTTT dTdT. Primers used to construct OGT–rescue plasmids were 5’-CGG AAC TTTGCA GTA GCT TGG AGC AAC CT TGG TG TGT GTT-3’.

For plasmid transfection, cells were seeded at 50–60% confluence/10 cm² Petri dish and transfected with 7.5 µg of plasmid DNA using FuGENE 6 according to the manufacturer’s instructions for immunoprecipitation (IP) experiments.

**In vitro kinase assay**

Chk1 in vitro kinase assay was performed as described previously. Briefly, recombinant Chk1 kinase was purchased from R&D Systems (catalog no. 1630-KS), incubated with purified GST–OGT with 1 M HEPES (pH 7.4), 1 M MgCl₂, 1 M dithiothreitol, 0.1 M Na₃VO₄, 0.1 M ATP or 1 µCi of [γ-³²P]ATP. After 20 min at 30 °C, reactions were stopped by the sample buffer. Protein samples were separated by SDS-PAGE and phosphate incorporation was determined by phosphorimager.

**IP and immunoblotting**

IP and IB experiments were performed as described before (31). The following primary antibodies were used for IB: RL2 (1:500), anti-OGT (1:1000), anti-Chk1 (1:1000), anti-β-actin (1:1000), anti-OGT–pSer20 (1:1000), anti-vimentin (1:1000), anti-vimentin–pSer71 (1:1000), anti-Myc (1:5000), anti-HA (1:5000), and anti-FLAG M2 (1:5000) (Sigma). Peroxidase-conjugated secondary antibodies were from Jackson ImmunoResearch Laboratories. Blotted proteins were visualized using the ECL detection system (Amersham Biosciences). Signals were detected by a LAS-4000, and analyzed using Multi Gauge (Fujifilm).

**Acknowledgments**—We thank Drs. Huadong Pei and Xiaochun Yu for plasmids, Dr. Huiqiang Lou for support, and members of the Xu lab for helpful discussion and comments on the manuscript.

**References**

1. Hart, G. W., Slawson, C., Ramirez-Correa, G., and Lagerlof, O. (2011) Cross talk between O-GlcNAcylation and phosphorylation: Roles in signaling, transcription, and chronic disease. *Annu. Rev. Biochem.* **80**, 825–858

2. Wang, Z., Gusec, M., and Hart, G. W. (2008) Cross-talk between GlcNAcylation and phosphorylation: Site-specific phosphorylation dynamics in response to globally elevated O-GlcNAc. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 13793–13798

3. D’Avino, P. P., Giancanti, M. G., and Petronczki, M. (2015) Cytokinesis in animal cells. *Cold Spring Harb. Perspect. Biol.* **7**, a015834

4. Wang, Z., Udeshi, N. D., Slawson, C., Compton, P. D., Sakabe, K., Cheung, W. D., Shabanowitz, J., Hunt, D. F., and Hart, G. W. (2010) Extensive crosstalk between O-GlcNAcylation and phosphorylation regulates cytokinesis. *Sci. Signal* **3**, ra2

5. Slawson, C., Lakshmanan, T., Knapp, S., and Hart, G. W. (2008) A mitotic GlcNAcylation/phosphorylation signaling complex alters the posttranslational state of the cytoskeletal protein vimentin. *Mol. Biol. Cell* **19**, 4130–4140

6. Lowery, J., Kuczmarski, E. R., Herrmann, H., and Goldman, R. D. (2015) Intermediate filaments play a pivotal role in regulating cell architecture and function. *J. Biol. Chem.* **290**, 17145–17153

7. Tsuchimura, K., Ogawa, M., Takeuchi, Y., Imajoh-Ohmi, S., Ha, M. H., and Inagaki, M. (1994) Visualization and function of vimentin phosphorylation by cdc2 kinase during mitosis. *J. Biol. Chem.* **269**, 31097–31106

8. Yamaguchi, T., Goto, H., Yokoyama, T., Silljé, H., Hanisch, A., Uldschmid, A., Takai, Y., Oguri, T., Nigg, E. A., and Inagaki, M. (2005) Phosphorylation by Cdk1 induces Plk1-mediated vimentin phosphorylation during mitosis. *J. Cell Biol.* **171**, 431–436

9. Goto, H., Yasui, Y., Kawajiri, A., Nigg, E. A., Terada, Y., Tatsuka, M., Nagata, K., and Inagaki, M. (2003) Aurora-B regulates the cleavage furrow-specific vimentin phosphorylation in the cytokinetic process. *J. Biol. Chem.* **278**, 8526–8530

10. Goto, H., Kosako, H., Tanabe, K., Yanagida, M., Sakurai, M., Amano, M., Kaibuchi, K., and Inagaki, M. (1998) Phosphorylation of vimentin by Rho-associated kinase at a unique amino-terminal site that is specifically phosphorylated during cytokinesis. *J. Biol. Chem.* **273**, 11728–11736

11. Yasui, Y., Goto, H., Matsu, S., Manser, E., Lim, L., Nagata, K.-I., and Inagaki, M. (2001) Protein kinases required for segregation of vimentin filaments in mitotic process. *Oncogene* **20**, 2868–2876

12. Matsuyama, M., Tanaka, H., Inoko, A., Goto, H., Yonemura, S., Kobori, K., Hayashi, Y., Kondo, E., Itohara, S., Izawa, I., and Inagaki, M. (2013) Defect of mitotic vimentin phosphorylation causes microcephaly and cata- ract via aneuploidy and senescence in lens epithelial cells. *J. Biol. Chem.* **288**, 35626–35635

13. Tanaka, H., Goto, H., Inoko, A., Makihara, H., Enomoto, A., Horimoto, K., Matsuyama, M., Kurita, K., Izawa, I., and Inagaki, M. (2015) Cytokinetic failure-induced tetraploidy develops into aneuploidy, triggering skin aging in phosphorym-en-deficient mice. *J. Biol. Chem.* **290**, 12984–12998

14. Peddibhotla, S., Lam, M. H., Gonzalez-Rimbau, M., and Rosen, J. M. (2009) The DNA-damage effector checkpoint kinase 1 is essential for chromosome segregation and cytokinesis. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 5159–5164

15. Mackay, D. R., and Ullman, K. S. (2011) A phospho-proteomic screen identifies substrates of the checkpoint kinase Chk1. *Genome Biol.* **12**, R78

16. Dey, A., Seshasayee, D., Noubade, R., French, D. M., Liu, J., Chauri shiya, M. S., Kirkpatrick, D. S., Pham, V. C., Lill, J. R., Bakalarski, C. E., Wu, J., Phu, L., Katavolos, P., LaFave, L. M., Abdul Wahab, O., et al. (2012) Loss of

**Author contributions**—J. Li wrote the manuscript. J. Li and X. X. designed the project and analyzed the data. Z. L., X. L., S. N., Q. G., and J. Liao performed the experiments. All authors reviewed and approved the manuscript.
the tumor suppressor BAP1 causes myeloid transformation. *Science* **337**, 1541–1546

18. Yang, Y., Yin, X., Yang, H., and Xu, Y. (2015) Histone demethylase LSD2 acts as an E3 ubiquitin ligase and inhibits cancer cell growth through promoting proteasomal degradation of OGT. *Mol. Cell* **58**, 47–59

19. Xu, Q., Yang, C., Du, Y., Chen, Y., Liu, H., Deng, M., Zhang, H., Zhang, L., Liu, T., Liu, Q., Wang, L., Lou, Z., and Pei, H. (2014) AMPK regulates histone H2B O-GlcNAcylation. *Nucleic Acids Res.* **42**, 5594–5604

20. Bullen, J. W., Balsbaugh, J. L., Chanda, D., Shabanowitz, J., Hunt, D. F., Neumann, D., and Hart, G. W. (2014) Cross-talk between two essential nutrient-sensitive enzymes: O-GlcNAc transferase (OGT) and AMP-activated protein kinase (AMPK). *J. Biol. Chem.* **289**, 10592–10606

21. Zhong, J., Martinez, M., Sengupta, S., Lee, A., Wu, X., Chaerkady, R., Chatterjee, A., O’Meally, R. N., Cole, R. N., Pandey, A., and Zachara, N. E. (2015) Quantitative phosphoproteomics reveals crosstalk between phosphorylation and O-GlcNAc in the DNA damage response pathway. *Proteomics* **15**, 591–607

22. Lima, V. V., Giachini, F. R., Carneiro, F. S., Carvalho, M. H., Fortes, Z. B., Webb, R. C., and Tostes, R. C. (2011) O-GlcNAcylation contributes to the vascular effects of ET-1 via activation of the RhoA/Rho-kinase pathway. *Cardiovasc. Res.* **89**, 614–622

23. Kim, D. H., Seok, Y. M., Kim, I. K., Lee, I. K., Jeong, S. Y., and Jeongh, N. H. (2011) Glucosamine increases vascular contraction through activation of RhoA/Rho kinase pathway in isolated rat aorta. *BMB Rep.* **44**, 415–420

24. Niu, Y., Xia, Y., Wang, J., and Shi, X. (2017) O-GlcNAcylation promotes migration and invasion in human ovarian cancer cells via the RhoA/ROCK/MLC pathway. *Mol. Med. Rep.* **15**, 2083–2089

25. Shi, A. M., Tao, Z. Q., Li, R., Wang, Y. Q., Wang, X., and Zhao, J. (2016) Vimentin and post-translational modifications in cell motility during cancer[em]a review. *Eur. Rev. Med. Pharmacol. Sci.* **20**, 2603–2606

26. Pan, T. L., Wang, P. W., Huang, C. C., Yeh, C. T., Hu, T. H., and Yu, J. S. (2012) Network analysis and proteomic identification of vimentin as a key regulator associated with invasion and metastasis in human hepatocellular carcinoma cells. *J. Proteomics* **75**, 4676–4692

27. Kidd, M. E., Shumaker, D. K., and Ridge, K. M. (2014) The role of vimentin intermediate filaments in the progression of lung cancer. *Am. J. Respir. Cell Mol. Biol.* **50**, 1–6

28. Whipple, R. A., Balzer, E. M., Cho, E. H., Matrone, M. A., Yoon, J. R., and Martin, S. S. (2008) Vimentin filaments support extension of tubulin-based microtubules in detached breast tumor cells. *Cancer Res.* **68**, 5678–5688

29. Jiu, Y., Peränen, J., Schaible, N., Cheng, F., Eriksson, J. E., Krishnan, R., and Lappalainen, P. (2017) Vimentin intermediate filaments control actin stress fiber assembly through GEF-H1 and RhoA. *J. Cell Sci.* **130**, 892–902

30. Chen, Q., Chen, Y., Bian, C., Fuji, R., and Yu, X. (2013) TET2 promotes histone O-GlcNAcylation during gene transcription. *Nature* **493**, 561–564

31. Tian, J., Tian, C., Ding, Y., Li, Z., Geng, Q., Xiahou, Z., Wang, J., Hou, W., Liao, J., Dong, M. Q., Xu, X., and Li, J. (2015) Aurora B-dependent phosphorylation of Ataxin-10 promotes the interaction between Ataxin-10 and Plk1 in cytokinesis. *Sci. Rep.* **5**, 8360