The biological mechanisms for maintaining the basal level of p53 in normal cells require nuclear exclusion and cytoplasmic degradation. Here, we showed that Jab1 facilitates p53 nuclear exclusion and its subsequent degradation in coordination with Hdm2. p53 was excluded from the nucleus in the presence of Jab1; this exclusion was prevented by leptomycin B treatment. Nuclear export of p53 was accompanied by a decrease in the levels of p53, as well as of its target proteins, which include p21 and Bax. Domain analyses of Jab1 showed that the N-terminal domain, 1–110, was capable of inducing cytoplasmic translocation of p53. Furthermore, 110–191 was required to facilitate the degradation of p53. Neither of these mutants incorporated into the CSN complex, indicating that Jab1 could affect the levels of p53 independent of intact CSN complex. Conversely, Jab1 was incapable of translocating and degrading two p53 mutants, W23S and 6KR, neither of which could be modified by Hdm2. Moreover, Jab1 did not affect the cellular localization or protein levels of p53 in p53 and Hdm2 double-null mouse embryo fibroblasts. We further observed that the ablation of endogenous Jab1 by small interfering RNA prevented Hdm2-mediated p53 nuclear exclusion. Under stressed conditions, which could sequester Hdm2 in its inactive state, Jab1 did not affect p53. Our studies implicate that Jab1 is required to remove post-translationally modified p53 and provide a novel target for p53-related cancer therapies.

p53, which is known as “the genome gatekeeper,” is a transcriptional factor that functions as a tumor suppressor (1, 2). p53 is activated in response to various oncogenic stresses and, thus, plays a key role in cancer prevention by maintaining genomic integrity through cell cycle arrest and/or apoptotic cell death (3).

Under normal conditions, p53 is maintained at low steady-state levels by its rapid turnover, which is mediated by ubiquitination followed by proteasome-dependent proteolysis (2, 4, 5). Among the various E3 ubiquitin ligases of p53, which include COP1, Pirh2, p300, and Cullin complex, Hdm2 is considered to be the most essential factor in p53 homeostasis (6–9). Studies examining the interaction between Hdm2 and p53 have proposed several models for Hdm2-facilitated p53 ubiquitination and the subsequent fates of p53 (4). One model suggests that Hdm2 and p53 form complexes, which are subsequently shuttled into the cytoplasm and degraded (10, 11). Interestingly, Hdm2 and p53 mutants, both of which are defective in nuclear localization, also induce p53 ubiquitination and degradation, which suggests that Hdm2 can be localized to the cytoplasm independently of p53 and can induce p53 ubiquitination and degradation in the cytoplasm (12). However, some reports have proposed that the nucleocytoplasmic shuttling of Hdm2 is not required for p53 cytoplasmic degradation to occur. This was based on evidence that Hdm2 mutants that are defective in cytoplasmic localization are still capable of degrading p53 (13, 14). Furthermore, monoubiquitinated p53 is excluded from the nucleus without Hdm2, which suggests that the unmasking of nuclear export signals (NES) might lead to p53 nuclear export (15, 28). However, p53 nuclear export still requires a combination of many factors including CRM1, RanGTP, and other factors (11).

Jun activation domain-binding protein 1 (Jab1)/CSN5 is the fifth member of the CSN complex group, and was first discovered as a transcriptional activator of c-Jun and JunD (16). It was later found to be a regulator of various factors including p27Kip1, cyclin E, Smad family, and SCF ubiquitin ligases (17–22). In particular, the CSN complex targets p53 for its phosphorylation and facilitates its degradation via the ubiquitin system (23). Additionally, Jab1 knock-out mice had higher p53 and p27 levels, suggesting the essential role of Jab1 in their homeostasis (24).

This study showed that Jab1 is involved in p53 cytoplasmic localization, which subsequently leads to the degradation of p53. Several regulatory pathways have been proposed to control these processes. These results suggest that the maintenance of p53 homeostasis requires the presence of Jab1, which may be another useful target for p53-related cancer therapies.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—pCMV/FLAG-Jab1, pcDNA3/FLAG-HDM2, and pCMV/HDM2 were kindly provided by K. W. Kim (National University of Seoul), E. J. Choi (University of Korea), and Y. P. Zhang (University of North Carolina, respectively). pcDNA3/HA-p53W23S was a generous gift from C. G. Maki (University of Chicago). pcDNA/p53-6KR was kindly provided by D. P. Lane (University of Dundee). pcDNA/Hsp70 was used for Hsp70 expression. pcDNA3/HA-Jab1 and pCS3-MT-BX(6xMyc)/Jab1 were

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8 This work was supported by 21C Frontier Functional Human Genome Project Grant FG05-22-02, Brain Research Center Frontier Grant M103KV1001805KZ201-01830 from Ministry of Science and Technology, and Science Research Granters R11-2000-080-09005-0 and R11-2002-000-00445-0 from the Korea Science and Engineering Foundation, and Simrnan Grant KR1-2002-003-E0016 from Sungkyunkwan University. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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4 The abbreviations used are: NES, nuclear export signals; Jab1, Jun activation domain-binding protein 1; CSN, COP9 signalosome; JAMM, Jab1/MPN/Mov34 metalloenzyme; HA, hemagglutinin; LMB, leptomycin B; MEF, mouse embryonic fibroblast; GFP, green fluorescent protein; DAPI, 4,6-diamidino-2-phenylindole; WCE, whole cell extract; siRNA, small interfering RNA.
generated by subcloning of the EcoRI fragment from pGEX-4T-1/\textit{jab1} into pcDNA3/HA (Invitrogen) and pCS3-MT-BX (6xMyc) (a generous gift from J. H. Ahn, Sungkyunkwan University). pcDNA3/HA/\textit{jab1} deletion mutants, 1–110, 110–191, and 1–191, were prepared by PCR using pcDNA3-HA/\textit{jab1} as a template and the following primers: 5′-CCGGAATTCATGGCTGCTGCGTATGAGTAT-3′ (forward) and 5′-CCGGAATTCCTACTTTGGATATGTCCTAAAGG-3′ (reverse, 1–110) or 5′-CCGGAATTCCTACTTTGGATATGTCCTAAAGG-3′ (reverse, 1–191) or 5′-CCGGAATTCATGGCTGCTGCGTATGAGTAT-3′ (reverse, 1–191). The PCR products were digested with EcoRI/Xhol or EcoRI and cloned into the corresponding sites of the plasmids of pcDNA-3/HA and pCS3-MT-BX (6xMyc). All constructs were confirmed by enzyme digestion and DNA sequence analyses. The pEGFP-C2 vector (Clontech) was used as a transfection control.

\textbf{Cell Culture and DNA Transfection—\textit{p53} null and \textit{p53/Mdm2} double knock-out MEFs were kindly provided by G. Lozano (University of Texas). The human cancer cell lines H1299, 293T, SK-N-SH, and U2OS, as well as the \textit{p53} null and \textit{p53/Mdm2} double knock-out MEFs, were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Invitrogen). The transient transfections were performed using the Lipofectamine Plus reagent (Invitrogen) according to the instructions of the manufacturer.}

\textbf{Antibodies and Chemicals—\textit{Jab1} (FL-334), Mdm2 (SMP-14), \textit{p53} monoclonal (DO-1), \textit{p53} polyclonal (FL-393), HA monoclonal (E-7), HA polyclonal (Y-11), p21 polyclonal (C-19), Bax polyclonal (N-20), and GFP (FL) antibodies were purchased from Santa Cruz Biotechnology. The FLAG (M2) and actin antibodies were purchased from Sigma.}
Hsp70 antibody (SPA810) was purchased from Stressgen. The Alexa Fluor 488 anti-mouse and Alexa 594 anti-rabbit antibodies were obtained from Alexa. The anti-c-myc monoclonal antibody (9E10) was purchased from Roche Diagnostics. CSN1 (ab10413), CSN2 (ab10426), COPS3 (ab12321), COPS4 (ab12322), and CSN7b (ab11895) antibodies were acquired from Abcam. LLnL, MG132, leptomycin B, DAPI (4,6-diamidino-2-phenylindole), propidium iodide, etoposide, and H$_2$O$_2$ were purchased from Sigma.

Immunofluorescence Staining—The cells were plated in 6-well plates with coverslips. After transfection, the cells were fixed with a 4% paraformaldehyde solution for 15 min at 25°C and washed. They were then permeabilized with 0.5% Triton X-100 in phosphate-buffered saline for 8.
15 min. After blocking with 5% bovine serum albumin (Santa Cruz Biotechnology) in phosphate-buffered saline for 30 min, the cells were incubated with the specific primary antibody overnight at 25 °C, followed by incubation with Alexa Fluor 488 anti-mouse or Alexa 594 anti-rabbit antibodies for 1 h at 25 °C. The cells were stained with DAPI for 7 min. The slides were analyzed using confocal or immunofluorescent microscopy (Carl Zeiss Vision, LSM510, Axioskop 2, and 5203 Axiophot II, respectively).

Biochemical Analyses—Immunoprecipitation and Western blot analysis were carried out as follows. At 48 h after transfection, the cells were harvested in a lysis buffer (150 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 0.5% Triton X-100, 0.1% sodium deoxycholate, 50 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, and 100 X protease inhibitor mixture). WCE (whole cell extracts) were incubated with the target antibody for 2 h at 4 °C. A/G beads (Roche) were added to the lysates, incubated for 2 h at 4 °C, precipitated, and washed three times in a lysis buffer. The bound proteins were dissolved in the sample buffer and resolved by SDS-PAGE. The resolved proteins were transferred to a nitrocellulose membrane (Amersham Biosciences), blotted with the primary antibody, and incubated with horseradish peroxidase anti-rabbit or anti-mouse IgG (Zymed Laboratories Inc.) for 1 h at 25 °C. Antibody binding was detected using an ECL system (Amersham Biosciences).

The luciferase activities were measured using a Dual Luciferase assay kit (Promega) according to the instructions of the manufacturer. The Renilla luciferase reporter plasmid, pRL-CMV, was used as an internal control.

The levels of endogenous Jab1 were decreased using Jab1 siRNA (5'-GCUCAGAGUAUCGAUGAAA-3') oligonucleotides with 3'-dT-dT overhangs, and were synthesized by Qiagen-Xeragon. Control siRNA in experiments refers to a mixture of scrambled siRNA oligonucleotides (Qiagen-Xeragon). The cells were transfected with 200 nM siRNA using Oligofectamine according to the instructions of the manufacturer (Invitrogen).

RESULTS

Jab1 Induces the Nuclear Exclusion and Degradation of p53—Because Jab1 is known to be a nuclear/cytoplasmic shuttle protein and interacts with p53, the effects of Jab1 on p53 cytoplasmic localization were first examined in H1299 and U2OS cells (17). Approximately 80% of the p53
expressed in H1299 was localized to the nucleus, whereas Jab1 was located in both the nucleus and the cytoplasm (Fig. 1A, panels 1–4, and graph). However, ~60% of cells simultaneously expressing p53 and Jab1 displayed both the cytoplasmic and nuclear localization of p53 (Fig. 1A, panels 5–8, graph). When leptomycin B (LMB), a CRM1 inhibitor, was added, it blocked Jab1-facilitated p53 nuclear export, which implied that this process requires the CRM1-dependent pathway (Fig. 1A, panels 9–12) (11).

It has been established that endogenous p53 is mainly expressed in the nuclei of U2OS cells (Fig. 1B, panels 1 and 2) (13, 14). Upon overexpression of Jab1, there was a significant reduction in p53 levels in the nuclei of 85% of cells expressing endogenous Jab1 (Fig. 1B, panels 5–7). This reduction was completely blocked by LMB (Fig. 1B, panels 8–10). These observations suggest that Jab1 might facilitate p53 nuclear exclusion and degradation.

When the biochemical effects of Jab1 on the stability of p53 were tested, we observed that the overexpression of Jab1 induced a reduction in the levels of exogenous p53 in H1299 (Fig. 2A). Consistent with these findings, exogenous Jab1 also decreased the level of p21 and Bax, both target proteins of p53 (Fig. 2B). It also suppressed the p53-dependent activation of synthetic reporter genes, including p21Waf1, Bax, and PG13 (Fig. 2C). Moreover, Jab1 facilitated the degradation of endogenous p53, which was blocked in the presence of MG132, a proteasome inhibitor (Fig. 2D). Because Jab1 has been implicated in several transcriptional activities as a cofactor, we tested whether the exogenous Jab1 might affect the mRNA levels of p53 (16, 17, 19). When we performed reverse transcriptase-PCR using p53 or glyceraldehyde-3-phosphate dehydrogenase primers, mRNA levels of p53 were not affected by Jab1 (Fig. 2D, lanes 1 and 2). These data imply that Jab1 promoted p53 degradation via the 26 S proteasome-mediated pathway. Because our data implies that the degradation process of p53 is accelerated in the presence of Jab1, we further examined whether the ablation of endogenous Jab1 affects the homeostasis of p53 in U2OS cells. The sequence of Jab1 siRNA used in this study has been reported previously (19). The treatment of Jab1 siRNA decreased endogenous Jab1 by 90%. The ablation of endogenous Jab1 was accompanied by an increase of ~10-fold in the p53 levels within 72 h (Fig. 2E, lanes 1 and 2). Taken together, these results imply that Jab1 may promote CRM1-dependent nuclear exclusion and the subsequent cytoplasmic degradation of p53 in the 26 S proteasome-dependent pathway.

**The N-terminal Domain of Jab1 Is Required for the Translocation and Degradation of p53—**The direct interaction between p53 and Jab1 has been reported previously, and we confirmed the data using purified recombinant p53 and Jab1 proteins (data not shown) (22). We further examined the region of Jab1, which is responsible for p53 interaction, nuclear export, and degradation using 6xMyc-tagged wild-type Jab1 or its deletion mutants, 1–110, 1–191, and 110–191 (Fig. 3A). The mutants were constructed according to their abilities to be expressed in mammalian cells. The immunoprecipitation assays were carried out in the presence of MG132 to inhibit Jab1-facilitated degradation of p53. Immunoprecipitation data indicated that Jab1 and its deletion mutants, 1–110 and 1–191, bind to p53, whereas 110–191 did not (Fig. 3B and
C). This finding suggests that the 1–110 mutant is responsible for the physical interaction of Jab1 with p53 (22). Furthermore, 1–110 and 1–191 mutants were capable of inducing the nuclear export of p53, whereas the 110–191 did not have any effect (Fig. 3D, panels 5–16). Finally, Jab1 and 1–191, which contained an intact Jab1/MPN/Mov34 metallocenzyme motif (JAMM), were able to induce p53 degradation (Fig. 3E, lanes 2 and 4) (22, 25). This indicates that Jab1 contains two regions, one responsible for p53 interaction and nuclear exclusion, and another required for facilitating the degradation of p53.

Because Jab1 is one of the CSN complex, we examined whether intact CSN complex are required for Jab1-mediated regulatory networks on p53 (23). When immunoprecipitation assays were performed using Jab1 or its mutants, 1–110 or 1–191, we observed that, unlike Jab1, which bound to endogenous CSN1, 2, 3, 4, and 7b, the 1–110 and 1–191 deletion mutants did not interact with these CSN subunits. These findings suggest that the C-terminal region of Jab1 might be required for its association with other CSN subunits (Fig. 3F). Overall, the N-terminal domain of Jab1, 1–110, is responsible for the nuclear export of p53, whereas the domain including the intact JAMM domain is needed for p53 degradation. Additionally, it is likely that the Jab1-mediated translocation and degradation of p53 could be carried out independently of intact CSN complex.

**Hdm2 Is Required for Jab1-facilitated Nuclear Exclusion and Degradation of p53**—Because Hdm2 is known to be involved in the translocation and degradation of p53, we examined whether Jab1, which exhibits a similar outcome as that of Hdm2, could affect p53 independent of Hdm2 (4). For this, we employed two p53 mutants, W23S and 6KR, which retain intact p53 transcriptional activities (26, 27). W23S lost its ability to bind to Hdm2, whereas 6KR cannot be ubiquitinated by Hdm2 because all of its lysine sites are changed to arginine. Immunoprecipitation analysis showed that Jab1 could interact with both W23S and 6KR (Fig. 4A). Conversely, immunofluorescent and biochemical analyses showed that Jab1 was incapable of translocating or degrading W23S and 6KR (Fig. 4B). The effect of Hdm2 on the function of Jab1 was further identified using p53 null or p53/Mdm2 (mouse Hdm2) double knock-out mouse embryo fibroblasts (MEFs) (28). Whereas Jab1 was capable of inducing

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**FIGURE 6.** Jab1 is required for Hdm2-mediated p53 nuclear exclusion. A, H1299 cells were treated with the control or Jab1 siRNA. After 48 h, the endogenous levels of Jab1 and actin were detected using anti-Jab1 and anti-actin antibodies, respectively. B, H1299 cells pretreated with the control (panels 1–8) or Jab1 siRNA (panels 9–12) were transfected with the plasmids expressing HA-p53 (panels 1 and 2), Hdm2 (panels 3 and 4), or HA-p53 + Hdm2 (panels 5–12). The proteins were detected as described in the legend to Fig. 5A. C, the percentage of subcellular localization of p53 was determined by counting 200 cells. D, H1299 cells pretreated with the control (panels 1–3) or Jab1 siRNA (panels 4–6) were transfected with the plasmids expressing Hsp70. The proteins were detected using Hsp70 and Alexa Fluor 488 anti-mouse antibodies. E, the percentage of subcellular localization of Hsp70 was determined by counting 200 cells.

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**Jab1 Is Required for Maintaining p53 Homeostasis under Normal Conditions**
p53 nuclear export and degradation in p53 null MEFs, it had no effect on the cellular localization or degradation of p53 in p53/Mdm2 double-null cells (Fig. 5, A, panels 3–5 and 8–10, B, and C). Hdm2, on the other hand, was able to induce p53 nuclear export in both MEF cell types, as previously reported (Fig. 5, A, panels 11–13 and 16–18, B and C) (29).

The requirement of Hdm2 for Jab1-mediated p53 nuclear export and degradation prompted us to question whether Jab1 is also required for Hdm2-mediated p53 nuclear exclusion. Using Jab1-specific siRNA as described above, we observed that the endogenous levels of Jab1 were suppressed by 90% in H1299 cells (Fig. 6A). Approximately 70% of cells expressing both exogenous p53 and Hdm2 showed the nuclear/cytoplasmic distribution of p53, whereas the remainder showed nuclear localization (Fig. 6, B, panels 5–8, and C) (12). Conversely, the exogenous p53, which was co-expressed with Hdm2, was primarily located in the nucleus when Jab1 was ablated (Fig. 6, B, panels 9–12, and C). Using Hsp70, which is translocated into the cytoplasm by CRM1 independently of Hdm2, we further tested whether the ablation of Jab1 could affect the CRM1-mediated cellular localization of Hsp70 (30). The results showed that the ablation of Jab1 did not have any effect on the subcellular localization of exogenous Hsp70, whereas ~70% of p53 was located in the nucleus in the presence of Hdm2 as described above (Fig. 6, D and E, data not shown). These data suggest the specific role of Jab1 in p53 localization in coordination with Hdm2.

Overall, Jab1-facilitated p53 nuclear exclusion and degradation require the post-translational modification of p53 by Hdm2. More specifically, the binding of Jab1 to W23S or 6KR is not enough to induce p53 nuclear exclusion and degradation, which suggests that the Hdm2-mediated ubiquitination of p53 is required. Furthermore, Hdm2-mediated p53 nuclear export was not accomplished in the absence of Jab1, which indicated that Jab1 could play an essential role in the export of p53 into the cytoplasm.

Jab1-facilitated p53 Nuclear Export and Degradation Are Prevented under Stressed Conditions—We further examined what would happen to the Jab1-mediated p53 degradation pathway when the function of Hdm2 was inhibited by genotoxic stresses (31–33). It has been previously reported that Hdm2 is sequestered into the nucleolus by p14Arf or promyelocytic leukemia nuclear bodies under stressed conditions (32–34). Because genotoxic stresses negatively regulate the function of Hdm2, we expected that Jab1 might not have any effect on p53 nuclear export or degradation. As previously shown, the expression of Jab1 in the presence of p53 in H1299 and U2OS cells induced cytoplasmic localization and degradation of p53, respectively (Fig. 7, A, panels 1–3 and 10–12, and B, lanes 1 and 2). However, when treated with etoposide or H2O2, Jab1-mediated p53 nuclear export and degradation were inhibited in both cell lines, indicating that active Hdm2 might be required in this process (Fig. 7, A, panels 4–9 and 13–18, and B, lanes 3–6).

DISCUSSION

Recent studies have indicated that the levels of ubiquitination by Hdm2 are closely related to the nuclear or cytoplasmic localization of p53 (15). However, the regulatory mechanisms bridging the ubiquitination and nuclear export of p53 have not been fully addressed yet. This report suggests that p53 ubiquitination and its subsequent nuclear export and degradation might require the accessibility of p53 to Jab1 in addition to Hdm2. The Jab1-facilitated mechanism, however, requires several essential steps.

First of all, the ubiquitination of p53 by Hdm2 appears to be a prereq-
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usite for Jab1-promoted p53 nuclear exclusion. For example, the nuclear localization and degradation of the p53 mutants, W23S and 6KR, which could be neither ubiquitinated nor degraded by Hdm2, were not influenced by jab1 (Fig. 4). Furthermore, jab1 was unable to induce translocation and degradation of p53 in the absence of Hdm2 (Fig. 5). It seems that the interaction between p53 and Hdm2 preceding Jab1 would allow for the ubiquitination of p53, which would expose the nuclear export signal for subsequent subcellular translocation in a Jab1- and CRM1-dependent manner (Fig. 1) (29). Furthermore, our results suggest that a CSN complex-independent function of Jab1 might be associated with the nuclear exclusion of p53. This was highlighted by the two Jab1 mutants, 1–110 and 1–191, which did not associate with the intact CSN complex, but were capable of binding to p53 and inducing its cytoplasmic localization.

Notably, it was observed that the Jab1 deletion mutant, 1–191, which contained the JAMM motif, could induce p53 nuclear export and subsequent degradation in the same manner as the Jab1 wild-type, whereas 1–110 was only able to carry out the latter function. The JAMM motif is also found in RPN11 (regulatory particle number 11), a subunit of the 26 S proteasome that is involved in the ATP-dependent degradation of ubiquitinated substrates (25). More specifically, this motif has been known to participate in the regulatory mechanisms of ubiquitination and neddylation (25). Thus, it appears that the JAMM motif plays an essential role in p53 degradation. However, it is not yet clear whether other co-factors that connect Jab1 and its substrates to the 26 S proteasome complex are required.

It has been previously suggested that Jab1 connects p27 to CRM1 to facilitate p27 nuclear export (35). It is quite possible that Jab1 would function in a similar way for p53 as for p27. The presence of Jab1 induced the nuclear export and the subsequent cytoplasmic degradation of both proteins. However, several different regulatory pathways may exist that are involved in Jab1-mediated cytoplasmic translocation of p27 and p53. p27 does not have a NES, whereas p53 has its own. It has been suggested that Jab1 would provide p27 with the NES through direct interaction, which is required for CRM1 interaction and, thus, subsequent nuclear export of p27 (17, 35). This was further demonstrated by the use of a Jab1 mutant lacking NES, which inhibited Jab1-mediated nuclear export of p27 (35). On the other hand, our data indicated that the Jab1 mutant lacking NES (1–191) was still able to induce p53 localization (Fig. 3). These observations imply that the p53 NES is required for Jab1-mediated nuclear export to occur. In fact, a p53 mutant lacking NES was not translocated into the cytoplasm in the presence of Jab1 (data not shown). It is likely that the formation of a Jab1 and p53 complex might help to induce the exposure of the p53 NES to CRM1, which would accelerate the subsequent cytoplasmic translocation. Another possibility is that Jab1 functions as an adaptor that links CRM1 and p53 and positively increases the specificity of CRM1 toward p53. This process seems to be specific for p53, because Hsc70, which is also translocated into the cytoplasm by CRM1, was not affected by the ablation of Jab1 (Fig. 6) (30). However, it is still unclear how Jab1 might be directly involved in accelerating the translocation of p53 into the cytoplasm in a CRM1-dependent manner; further studies are required. Moreover, as Jab1-dependent p53 translocation requires the ubiquitination of p53 by Hdm2, it seems that the p53 nuclear export pathway can be seen as an extra regulatory pathway compared with that of p27.

What happens when cellular stresses occur? If DNA damage were to take place, the regulatory function of Hdm2 on p53 would be prevented during the early stages of the stresses, possibly through post-transla-
tional phosphorylation of both proteins and Hdm2 sequestration in the nucleolus by p14ARF or in the promyelocytic leukemia nuclear bodies (32–34). These inhibitions prevent the ubiquitination and subsequent degradation of p53. Consistently, the oncogenic stresses, which could damage DNA, prevented Jab1-mediated nuclear export and subsequent cytoplasmic degradation of p53 (Fig. 7). The inhibition of Hdm2 seems to prevent the modification of p53, a process that is essential for Jab1-mediated p53 nuclear exclusion and degradation (Fig. 5). However, we were not able to completely exclude the possibility that stress-induced post-translational modifications of Jab1 occur; this would inactivate the regulatory effects of Jab1 on p53.

Clinically, the overexpression of Jab1 in vivo has been implicated in various cancers, including those in the lung, pancreas, mouth, thyroid, breast, pituitary, ovary, and brain (36–43). Furthermore, the recent reports on Jab1-knock-out mice, which were embryonically lethal, showed increased p27 and p53 levels in the embryonic cells, which further supported our results (24). Therefore, Jab1 might function as a proto-oncogenic protein, the overexpression of which would negatively affect p53 and p27, either by reducing their levels or by removing them from the areas where their main regulatory functions are performed. Thus, Jab1 might be a novel target molecule in the development of drugs for the treatment of tumors that have erupted through disruptions of endogenous p53 homeostasis or through abnormal subcellular localization of p53.

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