Induction of Homologue of Slimb Ubiquitin Ligase Receptor by Mitogen Signaling*

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Vladimir S. Spiegelman‡§, Weigang Tang‡, Andrew M. Chan‡, Makoto Igarashi‡, Stuart A. Aaronson†, David A. Sassoon**, Masaru Katoh‡‡, Thomas J. Slaga‡, and Serge Y. Fuchs‡‡§§

From the ²AMC Cancer Research Center, Lakewood, Colorado 80214, ‡Department of Animal Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, (The Derald H. Ruttenberg Cancer Center and **Department of Biochemistry and Molecular Biology, Mount Sinai School of Medicine, New York, New York 10029, and ‡§Genetics Division, National Cancer Center Research Institute, Tokyo 104-0045, Japan

Homologue of Slimb (HOS) is the substrate-recognizing component of the SCPHOS-Roc1 E3 ubiquitin protein ligase. This ligase mediates ubiquitination of the inhibitor of NF-κB transcription factor (IκB). We have found that HOS is highly expressed in a number of human cancer cell lines. The rates of the HOS gene transcription as well as HOS mRNA and protein levels were up-regulated in cells treated with mitogens or transfected with the inducers of mitogen-activated protein kinase pathway. Conversely, mitogen withdrawal strikingly reduced HOS levels during differentiation of mouse myoblasts. Activators of mitogen-activated protein kinase accelerated IκBα degradation and increased NF-κB transcriptional activity. Inhibition of HOS function via expression of dominant negative HOS (HOSdn) initiated mouse myoblast differentiation and prevented Ras-mediated acceleration of IκBα degradation as well as NF-κB trans-activation and transformation of NIH3T3 cells. These data link the induction of HOS in proliferating cells with mitogen-signaling-dependent inhibition of cell differentiation and promotion of cell transformation.

Accelerated proliferation and enhanced survival appear to be the paramount attributes of tumor cells. The mitogen-activated protein kinase (MAPK)1 pathway is instrumental for both of these characteristics. MAPK signaling is activated in response to external proliferative stimuli (growth factors, phorbol esters, etc.) or expression of oncogenic proteins. Structural components and activities of this pathway are frequently altered in cancer cells. MAPK is induced by a number of canonical oncoproteins (including Ras, v-Raf, etc.). Its signaling cascade involves activation of serine/threonine protein kinase Raf and subsequent phosphorylation and activation of mitogen-activated extracellular signal-regulated kinases kinases (MEK1 and MEK2) and extracellular signal-regulated protein kinases (Erk1 and Erk2). Extracellular signal-regulated kinases activate specific transcription factors, thereby inducing de novo expression of genes whose products accelerate cell cycle progression (1). Among these factors is the nuclear factor κB (NF-κB), in which activation by mitogens is often observed in dividing cells and is essential for normal growth (for review, see Ref. 2).

Increased cell viability represents a byproduct of MAPK signaling that is favorable for individual cells. Activation of NF-κB by MAPK plays a central role in survival of cells undergoing malignant transformation (3). It occurs in cells transformed by several cellular and viral oncoproteins including Ras, Raf, and others. Enhanced constitutive activity of NF-κB is widely observed in human tumor cells and primary tumors (for review, see Refs. 2 and 4). Induction of NF-κB by a transforming Ras mutant is required for malignant transformation of NIH3T3 cells (5). Inhibition of NF-κB activity prevents Ras-mediated focus formation (6) and even may lead Ras-expressing cells to death via apoptosis rather than to accelerated growth (7).

In the absence of stress or infection, normal cells suppress basal NF-κB activity and inhibit expression of numerous NF-κB target genes by sequestering NF-κB outside the nucleus. This is achieved by expression of a family of inhibitory proteins (IκB). IκBs tightly bind to NF-κB dimers and mask the NF-κB domains, which are responsible for the nuclear localization and DNA binding. A number of external stimuli, including mitogens, stress, damage, and inflammatory cytokines can activate NF-κB via ubiquitin-dependent degradation of IκB, which requires phosphorylation of IκB on two critical serine residues by IκB kinases (for review, see Ref. 8).

Phosphorylated IκB can be recognized by two closely related F-box proteins, βTrCP/Fbw1a (9–12) or HOS/βTrCP2/Fbw1b (13, 14). Both these proteins target IκB for ubiquitination (followed by 26 S proteasome-dependent degradation) via recruitment of the SCPHOS/βTrCP-Roc1 E3 ubiquitin ligase (for review, see Ref. 15). Besides ubiquitination of IκB, these ligases are responsible for ubiquitination and degradation of specifically phosphorylated transcription factors ATF4 (activation transcription factor 4) (16) and β-catenin (for review, see Ref. 15) as well as NF-κB/NF-κB2 processing (17, 18). Endogenous levels of HOS and βTrCP are low (19–21), and their function can be easily disrupted by overexpression of dominant negative constructs (9–11, 14) or pseudosubstrates (22–24). Conversely, forced expression of HOS (14) or βTrCP (12) often results in
increased activity of a respective E3 ubiquitin ligase and accelerated degradation of its substrates. These data suggest that effectors that control the abundance of HOS or βTrCP play an important role in the regulation of NF-κB and β-catenin/Tcf signal transduction pathways.

Such a role has been recently demonstrated for induction of βTrCP in cells that express an oncogenic mutant of β-catenin (19). In this case, βTrCP-based ubiquitin ligase did not affect stability of a phosphorylation-deficient β-catenin mutant (due to the lack of specific phosphorylation) but did contribute to activation of NF-κB by targeting IκB ubiquitination and degradation. We have found that βTrCP is overexpressed in a number of colorectal tumors (19, 21) and suggested that elevated levels of βTrCP may play a role in NF-κB activation and enhanced survival of tumor cells. Interestingly, we found that some cell lines (e.g. NIH3T3) or tissues (e.g. mouse skin) hardly express any βTrCP (20, 25). Thus, we sought to investigate the regulation of HOS expression in NIH3T3 cells and to determine the levels of HOS in human tumors.

In this study, we have detected high levels of HOS expression in a number of human cancer cell lines. We have also found that proliferative stimuli induced HOS expression and that activation of the MAPK pathway was necessary and sufficient for HOS induction and concurrent activation of NF-κB-driven transcriptions. Maintenance of HOS levels in mouse myoblasts correlated with their proliferation and lack of differentiation. Moreover, the integrity of HOS function was indispensable for Ras-induced IκBα degradation, NF-κB activation, and transformation of NIH3T3 cells in vitro. Thus, we discussed the association between cell proliferation and HOS levels and the likely contribution of HOS induction by mitogens to cell differentiation and transformation and perhaps tumorigenesis.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfections**—All cells originated from the American Type Culture Collection except TIG normal human fibroblasts (a gift from H. Tahara) and normal epithelial prostate cells (purchased from Clonetics). All cells were cultured in the presence of antibiotics at 37 °C, 5% CO₂ and 95% humidity. NIH3T3 and TIG normal human fibroblasts were maintained in Dulbecco’s modified Eagle’s medium with 10% calf serum (Invitrogen). Murine C2C12 myoblast cells were cultured to proliferate in Dulbecco’s modified Eagle’s medium supplemented with 20% fetal bovine serum (HyClone) or induced to differentiate in 2% horse serum (Sigma) and insulin (10 μg/ml). All tumor cell lines were cultured in RPMI medium or a mixture of Dulbecco’s modified Eagle’s medium/F-12 (1:1) supplemented with 5–15% fetal bovine serum. Normal prostate epithelial cells, PrEC, were cultured in PrEGM-F12 medium/F-12 (1:1) supplemented with 5–15% fetal bovine serum. Normal prostate epithelial cells PrEC, were cultured in PrEGM-F12 medium/F-12 (1:1) supplemented with 5–15% fetal bovine serum. Normal prostate epithelial cells PrEC; 2°C, 5% CO₂ and 95% humidity. NIH3T3 and TIG normal human fibroblasts were maintained in Dulbecco’s modified Eagle’s medium with 10% calf serum (Invitrogen). Murine C2C12 myoblast cells were cultured to proliferate in Dulbecco’s modified Eagle’s medium supplemented with 20% fetal bovine serum (HyClone) or induced to differentiate in 2% horse serum (Sigma) and insulin (10 μg/ml). All tumor cell lines were cultured in RPMI medium or a mixture of Dulbecco’s modified Eagle’s medium/F-12 (1:1) supplemented with 5–15% fetal bovine serum. Normal prostate epithelial cells, PrEC, were cultured in PrEGM medium (Clonetics).

**Plasmids**—Constructs for expression of HOS and HOS (14), HOS promoter-driven luciferase reporter (21), H-Ras R12 (26), v-Raf (27), dominant negative MEK1 (28), FOG-1β (29), 2x8B-Luc, and GST-1xβ (30) were described previously. We created pCDNA3-HOS(33) by PCR-mediated mutagenesis, resulting in deletion of cDNA sequence encoding HOS amino acids 117–169. Constitutively active MEK1 (MEK1) (32) was made by introduction of three substitution mutations (F53L, S218E, S222E) in the wild type MEK1 cDNA.

**Antibodies, Immunoprecipitation, and Immunoblotting**—N-HOS rabbit antibody, which specifically recognizes HOS, is described elsewhere (21). The other antibodies used for this study were hemagglutinin (HA) rabbit antibody and myogenin antibody (M-225) from Santa Cruz, M2 FOG-1β antibody, and mouse monoclonal antibody against heavy chain of skeletal myosin from Sigma. Texas Red-conjugated anti-rabbit and fluorescein isothiocyanate-conjugated anti-mouse secondary antibodies were purchased (Jackson Immunoresearch Laboratories, Inc., West Grove, PA). Preparations of cell lysates, immunoprecipitation, immunoblotting, and immunocytochemistry procedures are described elsewhere (14, 31). Data were analyzed using Scion Image Software (version Beta 4.0.2).

**In Vitro Transformation Assays**—NIH3T3 cells were transfected by calcium phosphate precipitate technique as described (28). For each 100-mm plate, 60 ng of Ras expression vector was transfected along with the indicated quantity of HOS or Leu expression vectors or empty vector to normalize the total amount of transfected plasmids. Cells were fed every 3 days with Dulbecco’s modified Eagle’s medium supplemented with 5% calf serum, and the appearance of foci of transformed cells was counted 14 days after transfection. The procedure for semi-solid agar growth assay is described elsewhere (32). Northern Blot, Dot Blot, and Nuclear Run-on Analysis—Total RNA was isolated with Trizol reagent (Invitrogen). RNA samples were fractionated on an agarose gel, transferred to a nylon filter, and cross-linked with a UV cross-linker (Stratagene). Blots were hybridized with HOS-specific [α-32P]dCTP-labeled probes in QuickHyb buffer (Stratagene) supplemented with denatured salmon sperm DNA, stringently washed, and analyzed by autoradiography. Dot blot analyses of cDNAs from primary human tissues fixed to a nylon filter (the Matched Tumor/Normal Expression Array membrane, CLONTECH) were hybridized with the probes to HOS or ubiquitin according to the manufacturer’s recommendations. Nuclear run-on analysis was performed as previously described (19).

**RESULTS**

HOS Is Overexpressed in Tumor-derived Cell Lines and Primary Human Cancers—We determined the levels of HOS mRNA in human cancer cell lines and primary human cancer tissues. Steady state levels of HOS mRNA were increased in four of six cell lines derived from human prostate cancers when compared with normal epithelial prostate cell lines (Fig. 1A, lanes 4–7 versus lane 1). HOS expression was also elevated in one of three tissues from primary human prostate tumors when compared with apparently normal tissues (Fig. 1C, lane 2). We also observed an increase in HOS expression in five of five human breast cancer cell lines compared with a non-transformed MCF10a cell line (Fig. 1B, lanes 2–6 versus lane 1), and five of nine primary breast tumors (Fig. 1D, lanes 2, 4, 5, 6, and 8). In all, our findings indicate that HOS is overexpressed in a considerable fraction of human primary cancers and cancer cell lines.

**Mitogenic Stimuli Induce Expression of HOS**—To investigate a possible link between cell proliferation and HOS expression, we used NIH3T3 cells, which do not express detectable levels of βTrCP (20). Treatment of these cells with a well characterized mitogen and inducer of NF-κB, 12-O-tetradecanoylphorbol-13-acetate (TPA) (33), resulted in an increased rate of HOS transcription as measured in a nuclear “run-on” assay (Fig. 2A).
Mitogen Induce HOS Expression by Activation of the MAPK Pathway—Growth stimuli are known to initiate numerous signal transduction pathways, including activation of the Ras-Raf-MEK1-MAPK cascade (1). Previously, we observed that HOS levels were elevated in skin tumors induced in SENCAR mice by a two-stage experimental skin carcinogenesis protocol (25), which yields skin tumors harboring activating Ras mutations (35). To investigate whether activation of the MAPK pathway was indeed required for induction of HOS by mitogens, we pretreated cells with the pharmacological inhibitor of MEK1 (PD98059). PD98059 inhibited the induction of HOS by TPA (Fig. 3A) and epidermal growth factor. Furthermore, the expression of constitutively active Ras, Raf (v-Raf), or MEK1 (MEK1^{E305L}) led to a robust activation of luciferase reporter driven by the HOS promoter. This activation was partially inhibited by dominant negative MEK1 mutant (dnMEK1; Fig. 3B). These data indicate that activation of MAPK pathway is both necessary and sufficient for induction of HOS transcription. We have also observed that expression of MAPK pathway activators led to accumulation of endogenous HOS protein (Fig. 3C) and mRNA (data not shown). Because the transfection efficiency in our experiments was ~20–25%, it is likely that the real extent of HOS accumulation was underestimated. DnMEK1 not only abrogated the induction of HOS by Ras but also decreased basal HOS levels (Fig. 3C). These findings indicate that MAPK activities contribute to induction of HOS by mitogens as well as to the maintenance of basal HOS levels. We occasionally observed the appearance of slowly migrating HOS species, which might represent phosphorylated HOS, in the cells expressing MAPK-inducing constructs.

The Effect of MAPK Cascade Activation on Stability of IκBα and Activation of NF-κB—Accumulation of βTrCP in response to Wnt or c-Jun NH₂-terminal kinase activation resulted in an increased activity of SCF^{βTrCP} (19, 20). We tested whether an induction of HOS by MAPK may activate the respective HOS-dependent E3 ubiquitin ligase activity. Forced expression of Ras resulted in a noticeable acceleration in IκBα degradation, with the half-life changing from more than 80 min to less than 35 min (Fig. 4A and B). These data agree with the findings of Finco et al. (6), who observed a Ras-mediated increase in NF-κB binding to DNA in NIH3T3 cells. Whereas co-expression of HOS slightly promoted further Ras-induced IκBα degradation induced by Ras (Fig. 4A and B). Of note, the rate of IκBα degradation well correlated with the levels of HOS. The half-life of IκBα was reduced by half in the Ras-expressing cells (with an approximately 2-fold increase in HOS levels, Fig. 4B, inset) as compared with the cells, which did not express Ras. These data indicate that HOS is required for acceleration of IκBα degradation mediated by Ras.

Several reports indicate that activation of IκB phosphorylation by IκB kinases (IκB kinase β (36) or IκB kinase ε (33)) and Rsk1 (37, 38) contributes to TPA-, Ras-, or v-Raf-dependent activation of NF-κB. Thus, we tested the effects of the MAPK cascade activators on IκBα phosphorylation. Whereas expression of activated Ras or Raf increased the overall IκBα kinase activity, constitutively active MEK1 (MEK1^{E305L}) failed to do so (Fig. 4C, inset). Yet all these constructs were efficient in MAPK activation (as judged by immunoblotting with antibody against phospho-extracellular signal-regulated kinase; data not shown) as well as in stimulation of NF-κB transcriptional activity (Fig. 4C, black bars). This result suggests that activation of IκB phosphorylation by Ras or Raf may not entirely be mediated via MAPK activity. Co-expression of dominant negative HOS^{AP} did
not affect MAPK activation per se (data not shown) but inhibited the effect of all of the MAPK inducers on NF-κB transactivation (Fig. 4C, gray bars). As evident from the data presented in Fig. 4C, constitutively active MEK1 induced NF-κB in a HOS-dependent manner without further activation of IκB kinases. In all, our findings suggest that HOS induction contributes to activation of NF-κB by MAPK signaling.

**HOS Is Down-regulated during Myoblast Differentiation—** The undifferentiated status of C2C12 mouse myoblast cells in vitro is achieved by their maintenance in high serum conditions and requires elevated levels of NF-κB activity. Inhibition of NF-κB activity by a non-degradable IκBα super-repressor resulted in C2C12 cell differentiation even in the presence of proliferative stimuli (31). We tested whether the levels of HOS are altered upon myoblast differentiation. When placed in the low serum-containing medium, C2C12 cells started to express specific markers of differentiation (myogenin and skeletal myosin, Fig. 5, B and C) followed by development of characteristic morphological features of myocyte tubes (data not shown). At the same time, we observed a dramatic decrease in the levels of HOS mRNA (Fig. 5A) and protein (Fig. 5B). Of note, forced expression of dominant negative HOSΔN mutant per se led to C2C12 differentiation even in the high serum-containing medium (Fig. 5C), albeit with a lesser efficiency than observed with the IκBα super-repressor (data not shown). These findings together with earlier reports (31) suggest that maintenance of active HOS levels and NF-κB activity by mitogen signaling plays a key role in preventing myoblast differentiation.

**HOS Activities Are Essential for Ras Transformation of NIH3T3 Cells—** Activation of NF-κB by Ras or v-Raf is indispensable for malignant cell transformation induced by these oncogenes, and inhibition of NF-κB activity by the IκBα super-repressor can block this transformation (5–7). Thus, it was of interest to determine the effect of inhibiting HOS activities for NIH3T3 transformation. Transient expression of increasing concentrations of the HOS dominant negative construct (HOSΔN) led to a noticeable decrease in the ability of co-expressed Ras to induce NIH3T3 cells to form foci (Fig. 6, black bars) or grow in an anchorage-independent manner in soft agar (Fig. 6, gray bars). Similar results were obtained with another HOS mutant (HOSAN, data not shown) as well as with IκBαΔN super-repressor, used as a positive control. The latter data are in line with previously published results (6). These findings indicate that HOS activities are required for transformation of NIH3T3 cells by Ras oncogene.

**DISCUSSION**

Elevated activity of NF-κB is a hallmark of many malignant tumors. Activation of NF-κB in tumor cells is achieved by either expression of abnormal NF-κB proteins (e.g. viral v-Rel or constitutively nuclear and active NF-κB2B65) or by oncogene-mediated activation of NF-κB signaling. Activation of NF-κB supports transformation of cells induced by expression of various oncogenes (Ras, etc.), many of which activate MAPK signaling themselves, in turn elevating NF-κB activities for MAPK cascade as well as for oncogene-mediated cell transformation. Furthermore, HOS is overexpressed in a substantial fraction of human cancer cell lines (Fig. 1). We therefore conclude that induction of HOS by MAPK contributes to elevated NF-κB activities in quickly proliferating tumor cells.

In mammalian cells, HOS and βTrCP proteins are expressed at low levels (19–21). They target ubiquitination of those IκB molecules, which are phosphorylated at specific serine residues (8, 15). Both kinase activity and the levels of active ubiquitin...
Induction of HOS by Mitogens

**Fig. 4.** Effect of HOS induction by mitogens on SCF<sup>HOSt</sup> activities. A, stability of IκBα. NIH3T3 cells were transfected with pCMV4-FLAG-IκBα (0.5 μg; 1–4), Ras (0.5 μg; 2–4), and pcDNA3-HOS (1 μg; 3) or pcDNA-HOS<sup>ΔN</sup> (1 μg; 4). The total amount of transfected DNA was kept at 2 μg/60-mm plate by adding pcDNA3. 24 h after transfection the cells were incubated in the presence of cycloheximide (CHX; 60 μg/ml) for the indicated period of time and harvested. 50 μg of whole cell extracts were analyzed by immunoblotting with anti-FLAG antibody (M2, Sigma). The first lane of the upper panel represents the extract from the mock-transfected cells. Asterisks denote a nonspecifically recognized protein. A representative of four independent experiments is shown. B, a graphic depiction of the average result of four independent experiments similar to the one shown in A. Densitometric values presented are percent ratios of the amounts of FLAG-IκBα left at different time points to the initial (time point 0) quantity. The inset depicts levels of HOS proteins (denoted by arrows) in the corresponding samples at time point 0, as measured by immunoprecipitation-immunoblotting with HOS antibody. C, activation of NF-κB and overall IκBα phosphorylation. NIH3T3 cells were transfected with 2xIκBα-driven luciferase reporter (0.1 μg) and vectors for expression of β-galactosidase (0.05 μg), Ras (0.1 μg), v-Raf (0.1 μg), constitutively active MEK1 (MEK<sub>ΔN</sub>, 0.1 μg), and dominant negative HOS (HOS<sup>ΔN</sup>, 0.15 μg) as indicated. Luciferase activity was measured and depicted as in the legend to Fig. 3B. The inset shows the total IκB kinase activity in 5 μg of whole cell lysates from the cells transfected with 1 μg of indicated plasmids and harvested 24 h later (measured as described elsewhere) (51). Quantification of the data by phosphorimaging is reflected below the inset.

ligase may act as a limiting factor in IκB ubiquitination. Correspondingly, an increase in either IκB phosphorylation and/or HOS/βTrCP levels may be sufficient to activate NF-κB. Our previous data demonstrated that both induction of βTrCP and activation of IκB kinases add to NF-κB activation in response to cellular stress (20). Here we show that induction of HOS via MAPK pathway in response to mitogens contributes to activation of NF-κB in the cells, which do not express βTrCP. Expression of Ras or v-Raf elevates levels of HOS as well as activities of IκB kinases. Conversely, constitutively active MEK1 induces HOS and NF-κB activity without increasing the existing basal level of IκBα phosphorylation (Fig. 4C). These data, therefore, suggest that an increase in IκBα phosphorylation after expression of Ras or v-Raf is likely mediated by MAPK-independent signaling in NIH3T3 cells.

Activation of MAPK cascade robustly increased the transcription of HOS gene, but the extent of HOS protein accumulation was much more moderate (Figs. 2 and 3). Limited increase in the protein levels is most likely due to a rapid turnover of HOS mRNA and/or protein. The data showing that a 10-fold decrease in HOS mRNA during mouse myoblast differentiation resulted in ~25-fold reduction in HOS protein levels (Fig. 5, A and B) are within the similar line. It appears that the levels of HOS protein are tightly regulated in mammalian cells by both transcriptional and post-transcriptional mechanisms. This suggests that regulation of HOS abundance may significantly contribute to HOS function. Indeed, we demonstrated earlier that inhibition of HOS expression by the Wnt pathway in NIH3T3 cells leads to an inhibition of SCF<sup>HOSt</sup>-Roc1 E3 ubiquitin ligase activities (21). Moreover, in the present study we observe a remarkable correlation between the extent of changes in HOS levels and the acceleration of IκB degradation. Similarly, control of the βTrCP levels by Wnt or c-Jun NH<sub>2</sub>-terminal kinase pathways is essential in determination of SCF<sup>βTrCP</sup>-Roc1 E3 ubiquitin ligase activities (19, 20).

Activation of NF-κB by mitogens is required for normal cell growth (2). An inverse correlation between HOSt levels and NF-κB activities on one side and myoblast differentiation status on the other indicates that regulation of HOS levels may be linked with control of cell differentiation. Both dominant negative HOS and IκB constructs induce cell differentiation (Ref. 31 and Fig. 5C) and prevent outgrowth of Ras-expressing cells (Refs. 6 and 7 and Fig. 6). The effect of up-regulation of HOS by MAPK pathway on overall NF-κB activities may also be mediated via accelerated processing of p105, which is a known substrate for βTrCP/HOS-mediated ubiquitination (17). Although mitogen-dependent maintenance of NF-κB-dependent transcription might represent the major mechanism of HOS function in cell differentiation and transformation, it would be an oversimplification to consider it the only mechanism. We cannot rule out that some of the effects of MAPK-dependent HOSt up-regulation are mediated via other known (i.e. ATF4 (activation transcription factor 4) (16) or yet unknown HOS/βTrCP substrates in the regulation of a cell fate.)
Induction of HOS by Mitogens

Activation of the MAPK kinase signaling and NF-κB activities is among the most common traits of human cancers (2–4). One can predict that elevated HOS expression that is induced by MAPK and controls NF-κB may contribute to development of mammalian tumors. Indeed, when analyzing the Matched Tumor/Normal Expression Array (CLONTECH), we have detected increased HOS levels in a limited number of cDNA samples from primary human breast and prostate cancers compared with the samples from apparently normal tissues from the same patients (Fig. 1). Overexpression of HOS (or βTrCP) was also found in a fraction of human gastric (39) and colorectal (19, 21) cancers and in the experimentally induced mouse skin tumors (25). Because HOS and βTrCP target β-catenin for ubiquitination, it would be expected that β-catenin levels diminish in tumor cells with high expression of HOS or βTrCP. Conversely, we observed that in cells with activated MAPK, the share of phosphorylated β-catenin is decreased, whereas its total level is elevated (40). A decrease in phosphorylation of β-catenin that renders it insensitive to HOS/βTrCP-mediated ubiquitination (14) is most likely attributed to inhibition of GSK3β by MAPK (41, 42). Phosphorylation of β-catenin by GSK3β is often compromised in colorectal cancers because of either a mutation in phospho-acceptor region of β-catenin or defective recruitment of GSK3β to β-catenin (e.g. adenomatous polyposis coli (APC) mutations; for review, see Ref. 43). It is conceivable that induction of HOS/βTrCP in tumor cells not only confers elevation of NF-κB activities but also imposes a selective pressure for inhibition of β-catenin phosphorylation and its recognition by HOS/βTrCP-based SCF-Roc1 E3 ubiquitin ligases, thereby increasing the levels and activities of β-catenin.

Our data indicate that induction of HOS by mitogens is likely to contribute to cell transformation. Further comprehensive studies are required to determine whether a mitogen-dependent increase in HOS levels may actually contribute to the tumor development. Recent findings of several groups have already established the role of other members of the F-box family in the pathogenesis of human malignancies. Skp2, which targets ubiquitination of cyclin-dependent kinase inhibitor p27Kip1, is often overexpressed in tumor cells and implicated in lymphoma development (45, 46). Alterations in the Fbw7 protein (also termed hAgo/hCdc4) that target phosphorylation-dependent ubiquitination of cyclin E are also implicated in ovarian and breast cancers (47–49).

We have previously reported that inhibition of HOS/βTrCP function led to sensitization of human melanoma cells to the anti-tumor agents, including ionizing radiation and chemotherapeutic agent cisplatin (50). Further detailed studies of the expression pattern of HOS and its putative role in specific types and stages of human malignancies are required to decide whether HOS may be used as a potential target for the anticancer therapy.

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