Ubiquitination Regulates the Internalization, Endolysosomal Sorting, and Signaling of the Erythropoietin Receptor*‡§∗

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Ubiquitination is a common mechanism of down-regulation of mitogenic receptors. Here, we show that ubiquitination of the erythropoietin receptor (EpoR) at Lys256 is necessary and sufficient for efficient Epo-induced receptor internalization, whereas ubiquitination at Lys428 promotes trafficking of activated receptors to the lysosomes for degradation. Interestingly, EpoR that cannot be ubiquitinated has reduced mitogenic activities and ability to stimulate the STAT5, Ras/MAPK, and PI3K/AKT signaling pathways. We therefore propose that ubiquitination of the EpoR critically controls both receptor down-regulation and downstream signaling.

Erythropoietin (Epo)3 is the primary cytokine controlling red blood cell production, and its function is mediated through the Epo receptor (EpoR). EpoR lacks intrinsic enzymatic activities and relies on the cytosolic tyrosine kinase JAK2 for signal transduction. Epo binding to the EpoR activates JAK2, which in turn phosphorylates tyrosine residues in the EpoR cytoplasmic domain and initiates signaling cascades, including the STAT5, Ras/MAPK, and PI3K/AKT pathways, which ultimately result in the survival, proliferation, and differentiation of erythroid progenitor cells (1, 2).

In addition to eliciting downstream signaling, Epo binding results in rapid internalization of activated EpoR and its targeting to the lysosomes for degradation (3–5). Endocytosis of the EpoR critically controls the amplitude and duration of Epo signaling, as the cell-surface level of EpoR controls cellular Epo sensitivity (6), and endocytosis may lead to destruction of activated protein complexes to terminate signaling (7). We previously showed that Epo-induced EpoR internalization requires both JAK2 kinase activity and EpoR cytoplasmic tyrosine residues (3). Specifically, Tyr429, Tyr431, and Tyr479 in the EpoR cytoplasmic domain, by binding to the p85 regulatory subunit of PI3K, mediate Epo-dependent EpoR internalization through a PI3K activity-independent mechanism (3). JAK2 also stimulates ubiquitination of the EpoR, most likely through the E3 ubiquitin ligase B′-TrCP (β-transducin repeat-containing protein), which contributes to receptor endocytosis and down-regulation (5, 7). However, the exact processes controlled by ubiquitination of the EpoR are not entirely understood.

Here, we used cellular reconstitution with EpoR variants to show that ubiquitination of the EpoR itself promotes ligand-induced receptor internalization and lysosomal degradation. Of the five EpoR cytoplasmic lysine residues, ubiquitination of Lys256 mediates Epo-induced EpoR internalization, whereas ubiquitination of Lys428 promotes Epo-induced EpoR degradation. Surprisingly, a lysine-less EpoR in which all cytoplasmic lysine residues were replaced with arginines showed reduced mitogenic activity and attenuated activation of downstream signaling pathways. Therefore, ubiquitination of the EpoR regulates both its endocytosis and signal transduction.

EXPERIMENTAL PROCEDURES

Plasmid Constructs, Cell Lines, and Reagents—All EpoR mutants were generated in the pMX-IRES-GFP vector using the QuikChange site-directed mutagenesis kit (Stratagene) and verified by sequencing. JAK2 and JAK2KD were generated in the pMX-IRES-CD4 vector. γ2A cells stably expressing wild-type or mutant HA-EpoRs with JAK2 or JAK2KD were generated by infecting a 2A cell line that stably expresses the ectropic murine leukemia virus receptor with retroviruses encoding wild-type or mutant EpoR and JAK2 or JAK2KD. Populations of cells expressing GFP and CD4 above a predetermined level were isolated by FACS sorting (8, 9). Ba/F3 cells stably expressing wild-type or mutant HA-EpoRs were generated similarly with retroviruses encoding wild-type or mutant EpoR followed by FACS sorting. Antibodies were from the following sources: HA (Covance); JAK2 and phospho-JAK2 (Millipore); EEA1, LAMP2 (lysosomal-associated membrane protein 2), and STAT5 (Santa Cruz Biotechnology); actin (Sigma); phospho-AKT and phospho-ERK (Cell Signaling); phospho-STAT5 (BD Biosciences); and ubiquitin (Enzo Life Sciences). HA affinity resin was from Roche Applied Science and FLAG affinity resin was from Sigma. Horseradish peroxidase-coupled secondary antibodies and the ECL chemiluminescence system were from Amersham Biosciences. Lactacystin was from Boston Biochem.

Flow Cytometry and Data Analysis—Internalization of surface EpoR was measured by flow cytometry and analyzed as described previously (3). For signaling analysis, Ba/F3 cells stably expressing various EpoRs were starved for 4 h in RPMI media with 1% BSA, pretreated with 100 μM cytochalasin D, and incubated with 100 ng/ml Epo for 10 min. Internalized EpoR was measured by flow cytometry and analyzed as described previously (3).
for 60 min to reduce the maturing pool of receptors that could replenish the cell-surface pool of EpoR during Epo stimulation, and then stimulated with different concentrations of Epo for the indicated times. For kinetics experiments, 30 units/ml of Epo was used for induction. Subsequently, cells were fixed with 1.6% paraformaldehyde, permeabilized with methanol at 4 °C, and stained with allophycocyanin (APC)-conjugated phospho-STAT5 (1:30), phospho-ERK (1:100), or phospho-AKT (1:100) antibodies. Fluorescence was analyzed by flow cytometry on a FACSCalibur flow cytometer (BD Biosciences), and median fluorescence from 20,000 cells was used for data analysis.

**Immunoprecipitation and Immunoblotting**—γ2A cells stably expressing wild-type or mutant EpoRs were transfected with a plasmid expressing FLAG-tagged ubiquitin. 48 h later, cells were treated with 100 μM cycloheximide for 1 h to re-
duce the intracellular maturing pool of the EpoR. Subsequently, 20 μM lactacystin or 40 μM MG132 was added for 1 h followed by Epo induction. Cells were lysed with 1% Nonidet P-40 lysis buffer with protease inhibitors and 10 mM N-ethylmaleimide. Lysates were immunoprecipitated with HA or FLAG affinity resins. The precipitates were eluted with SDS sample buffer, separated by SDS-PAGE, transferred to nitrocellulose membranes, and probed with antibodies to HA, FLAG, or ubiquitin. Bound antibodies were detected by the ECL chemiluminescence system after incubation with horseradish peroxidase-coupled secondary antibodies. Cell lysates were also immunoblotted with antibodies to HA, phosphorylated JAK2, JAK2, or actin.

**Immunofluorescence**—γ2A cells stably expressing HA-EpoR or HA-5KR were seeded on glass coverslips. Coverslips were blocked and stained with anti-HA antibodies, fixed, permeabilized, and incubated with antibodies for EEA1 (early endosome antigen 1) as described in (3) except that rabbit anti-HA antibodies were used (1:125) when co-staining with LAMP2. Coverslips were incubated with LAMP2 antibodies (1:50), and the appropriate fluorescence conjugated secondary antibodies. Coverslips were mounted onto slides with a semipermanent Mowiol mounting medium (Calbiochem). Fluorescent images were taken on a Leica TCS SP5 confocal microscope with 63× oil objective lenses with numeric aperture of 1.25 N. Confocal section images were acquired by Leica acquisition software and analyzed with Image J and Adobe Photoshop.

**Glycosidase Digestion of EpoR and MTT Cell Proliferation Assay**—Glycosidase treatment of the various EpoR constructs with endoglycosidase H (New England Biolabs) or with peptide N-glycosidase F (New England Biolabs) and neuraminidase (New England Biolabs), and MTT cell proliferation assay were performed as described (9).

**RESULTS**

**Ubiquitination of EpoR Promotes Ligand-induced Internalization and Endocytosis**—To characterize the role of ubiquitinated EpoR in ligand-induced EpoR endocytosis and down-regulation, we engineered a mutant EpoR unable to be ubiquitinated, 5KR, in which all five cytoplasmic lysines of the receptor are replaced with arginines. We examined ubiquitination in γ2A cells stably co-expressing HA-EpoR and JAK2, HA-EpoR and kinase-deficient JAK2 (JAK2KD), or HA-5KR and JAK2. These cells also transiently express FLAG-tagged ubiquitin. After pretreatment with 20 μM lactacystin to inhibit proteasomal activities, cells were induced with Epo, and cell lysates were immunoblotted with anti-HA antibodies to detect HA-EpoR (Fig. 1A) or immunoprecipitated with anti-HA antibodies prior to anti-ubiquitin and anti-FLAG immunoblotting (Fig. 1B). EpoR was ubiquitinated in the presence of JAK2 but not in JAK2KD. Based on the apparent molecular weight that exceeds monoubiquitinated and multoubiquitinated (monoubiquitinated at multiple sites) EpoR, the EpoR is most likely polyubiquitinated. These results do not preclude the presence of low level mono- or multiubiquitinated EpoRs at abundance below our detection limit. Our observation is consistent with previous reports showing that EpoR is polyubiquitinated upon Epo stimulation in a JAK2 kinase activity-dependent manner (5, 10). In contrast, 5KR promptly activated JAK2 in response to Epo but did not become ubiquitinated (Fig. 1, A and B). Similar results were obtained in the presence of another proteasomal inhibitor, MG132 (supplemental Fig. 1).

To determine the effect of EpoR ubiquitination on receptor internalization, we used flow cytometry to determine the internalization kinetics. In these assays, cell-surface HA-tagged receptors were detected by staining nonpermeabilized cells with anti-HA antibodies followed by APC-conjugated anti-HA antibodies in nonpermeabilized γ2A (A) and Ba/F3 (B) cells. Median APC fluorescence was used to quantify cell surface expression of receptors, and results are normalized to that of the noninduced sample for each receptor. Relative levels of cell-surface 5KR and S437A in noninduced cells normalized to wild-type EpoR are shown on the right.

![Graph showing ubiquitination of EpoR](attachment:image.png)

**FIGURE 2.** EpoR cytoplasmic lysines are important for Epo-induced receptor internalization. Levels of cell-surface receptors at indicated time points after Epo induction were quantified by flow cytometry using APC-conjugated anti-HA antibodies in nonpermeabilized γ2A (A) and Ba/F3 (B) cells. Median APC fluorescence was used to quantify cell surface expression of receptors, and results are normalized to that of the noninduced sample for each receptor. Relative levels of cell-surface 5KR and S437A in noninduced cells normalized to wild-type EpoR are shown on the right.
The fate of cell-surface EpoR and 5KR upon Epo induction was investigated by confocal immunofluorescence microscopy. Nonpermeabilized cells were labeled with anti-HA antibodies prior to Epo induction. Cells were stimulated with Epo, fixed, permeabilized, and visualized with fluorescence-conjugated secondary antibodies. This approach thus followed the fate of only surface-tagged receptors prior to Epo treatment. HA-EpoR moved from the plasma membrane to an internal compartment that co-localized with the early endosomal marker EEA1 within 25 min after Epo treatment (Fig. 3A). At 33 min post-Epo stimulation, HA-EpoR staining partially co-localized with the LAMP2, a lysosomal marker, indicating that the EpoR had reached the lysosomes (Fig. 3B). Consistent with previous results that Epo-induced internalization of HA-5KR receptors was impaired, a substantial portion of HA-5KR receptors were not internalized and remained on the surface (Fig. 3, A and B). The fraction of HA-5KR that was internalized co-localized with EEA1 and LAMP2 (Fig. 3, A and B). Consistent with the defect in internalization, ligand-induced degradation of HA-5KR was impaired compared with HA-EpoR (Fig. 1C).

The E3 ubiquitin ligase β-TrCP has been implicated in EpoR ubiquitination, and mutation of the predicted β-TrCP recognition motif around Ser462 in the human EpoR abolishes β-TrCP binding and blocks EpoR ubiquitination (7). We therefore examined the internalization and down-regulation of the corresponding mutant murine EpoR, S437A. Epo-induced internalization of HA-S437A was less efficient, and its degradation was also defective (Figs. 1C and 2). Together, these results suggest that EpoR ubiquitination plays an important role in ligand-induced EpoR internalization and down-regulation.

To examine whether ubiquitination may be sufficient to target the EpoR to the endosomes, we fused to the C terminus of the HA-EpoR either ubiquitin or monoUb, the K48R/K63R ubiquitin variant that cannot support polyubiquitination, and examined the localization. We also examined EpoR fused to ubiquitin with K48R or K63R mutations. EpoR fused with ubiquitin (EpoR-Ub), ubiquitin with a K48R mutation (EpoR-UbK48R), ubiquitin with a K63R mutation (EpoR-UbK63R), or ubiquitin with K48R/K63R mutations (EpoR-monoUb) all had few mature receptors expressed on the cell surface even without stimulation, in contrast to wild-type EpoR (Fig. 4, A and B), indicating improper processing. Therefore, whether ubiquitination of the EpoR is sufficient to trigger internalization is not clear.

Ubiquitination of Lys256 Mediates Epo-induced EpoR Internalization, Whereas Ubiquitination of Lys428 Regulates EpoR Degradation—To determine which of the five lysine residues are responsible for EpoR internalization, ubiquitination, and down-regulation, we generated cells that express EpoR variants in which individual lysine was replaced with arginine. These mutated receptors were examined for Epo-induced

FIGURE 3. 5KR is defective in sorting to early endosomes and lysosomes. HA-EpoR or HA-5KR receptors were labeled with anti-HA antibodies prior to Epo stimulation for 25 (A) or 33 min (B). Cells were fixed and immunostained with anti-EEA1 (A) or anti-LAMP2 (B) antibodies followed by appropriate fluorescence-conjugated secondary antibodies. Representative confocal images (single section) are presented. Original magnification 63× (Leica TCS SP5). Scale bars, 5 μm.
internalization and degradation. As shown in Fig. 5A, K256R resulted in defective internalization similar to 5KR, whereas K276R, K348R, K388R, or K428R had little effect. Similar results were also observed in Ba/F3 cells (data not shown). In addition, restoring Lys256 on the lysine-less background (R4K256) rescued receptor internalization (Fig. 5B). Moreover, R4K256 restored EpoR polyubiquitination upon stimulation (Fig. 5C). Therefore, ubiquitination of Lys256 is both necessary and sufficient for ubiquitin-based Epo-induced EpoR internalization. We also examined mutant EpoRs with single lysine replacement in Epo-induced down-regulation. As shown in Fig. 6A, only K428R but not mutations of other lysine residues resulted in a dramatic decrease in degradation upon stimulation. Consistent with this observation, the portion of internalized K428R receptors that co-localized with LAMP2 was reduced compared with wild-type EpoR (44 versus 65%). Therefore, although Lys428 internalizes normally, internalized receptors are not efficiently sorted to the lysosome for degradation. Furthermore, restoration of Lys428 on 5KR (R4K256) rescued receptor polyubiquitination (Fig. 6B) and restored normal Epo-dependent receptor degradation (Fig. 6C). In summary, these results suggested that upon Epo stimulation, ubiquitination at Lys256 promotes receptor internalization, whereas ubiquitination at Lys428 promotes endolysosomal sorting and degradation.

**EpoR Mutant That Cannot Be Ubiquitinated Has Dysregulated Signaling**—To assess the consequences of defective EpoR ubiquitination on signaling, we determined the mitogenic activities of wild-type EpoR or 5KR in the IL-3 dependent hematopoietic Ba/F3 cells. Ba/F3 cells stably expressing the different EpoRs at similar levels were grown in the absence of IL-3 but with various concentrations of Epo, and their mitogenic activity was measured. As shown in Fig. 7A, cells expressing 5KR grew normally in Epo above 1 unit/ml.
However, at lower Epo concentrations, the mitogenic activity of cells expressing 5KR was impaired. As controls, cells expressing all constructs grew similarly in IL-3 conditioned media (data not shown). Therefore, defective ubiquitination of the EpoR is associated with reduced mitogenic activities despite normal JAK2 activation.

The three main signaling pathways stimulated by Epo are the STAT5, Ras/MAPK, and PI3K/AKT pathways. To determine the mechanism underlying the mitogenic activity defect of 5KR, we examined the activation kinetics of these pathways using flow cytometry and fluorescence-conjugated antibodies specific to the active forms of STAT5, ERK, and AKT. Activation of all three proteins was maximal at 20 min after induction with saturating Epo concentrations (30 units/ml) (Fig. 7B). We thus examined activation of STAT5, ERK, and AKT by wild-type EpoR or 5KR at 20 min post-induction with various Epo concentrations. Consistent with the reduced mitogenic activity, 5KR showed Epo hyposensitivity in activating all three pathways compared with wild-type EpoR (Fig. 7C). Because endosomes have been implicated in amplifying signaling, we hypothesized that impaired sorting of activated 5KR receptors to endosomes may be responsible for the reduced signaling through the STAT5, ERK, or AKT pathways. In line with this hypothesis, K256R, defective in internalization, also exhibited Epo hyposensitivity in activating ERK and AKT as well as reduced mitogenic activities as compared with wild-type EpoR (Fig. 7, D and E).

Meyer et al. (7) showed that a mutant human EpoR lacking the β-TrCP binding site was hypersensitive to Epo. Consistent with their results, we also observed a small but reproducible degree of Epo hypersensitivity for the corresponding mutant murine EpoR, S437A, which contrasts to the hyposensitivity of the 5KR receptor (Fig. 7A). One possibility is that one or more signaling proteins in the activated EpoR complex other than EpoR itself may be substrates of β-TrCP, and their ubiquitination negatively regulates EpoR signaling. In summary, in addition to receptor internalization and trafficking, ubiquitination of the EpoR plays an important role in controlling downstream signaling.

**DISCUSSION**

Here, we show that ubiquitination of the EpoR is required for efficient internalization and lysosomal degradation. Two lysine residues, Lys256 and Lys428, in the EpoR cytoplasmic domain, both polyubiquitinated upon Epo stimulation, contribute to distinct steps in the endocytosis process. Specifically, Lys256 mediates Epo-induced receptor internalization, whereas Lys428 is not required for internalization but regulates Epo-induced receptor sorting to the lysosome and degradation. These results are consistent with recent evidence suggesting that HRS (hepatocyte growth factor-regulated tyrosine kinase substrate), a protein with a ubiquitin-interacting motif, plays a critical role in the transport of the ubiquitinated gp130 from the early endosome to the late endosome for degradation (14). The ubiquitin-proteasome machinery has also been implicated in transporting the prolactin receptor from

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**FIGURE 6. Ubiquitination of Lys428 promotes Epo-induced EpoR degradation.** A, K428R is defective in receptor degradation upon stimulation in γ2A cells. B, add-back of Lys428 on lysine-less EpoR (R4K428) but not 5KR becomes ubiquitinated upon Epo stimulation. An asterisk indicates a non-specific band. C, restoring Lys428 on lysineless EpoR (R4K428) rescues Epo-induced receptor degradation. DMSO, dimethyl sulfoxide; IP, immunoprecipitation; IB, immunoblot.
FIGURE 7. 5KR-expressing cells are hyposensitive to Epo and defective in activating three major downstream pathways. A, Ba/F3 cells stably expressing HA-5KR are less sensitive to Epo than those expressing wild-type EpoR. In contrast, cells expressing HA-S437A are hypersensitive to Epo. Mitogenic activities were measured by MTT assays after 48 h. Data for each construct are normalized to growth in IL-3 conditioned media. B, Ba/F3 cells stably expressing 5KR activated STAT5, MAPK, and AKT with similar kinetics compared with wild-type EpoR. Activation was assessed with 30 units/ml Epo over time using flow cytometry with APC-conjugated antibodies to phospho-STAT5, phospho-ERK, and phospho-AKT, respectively. Median APC fluorescence was used for analysis. Results were normalized to the maximal activation of wild-type EpoR. Data represent three independent experiments. C, Ba/F3 cells stably expressing 5KR are defective in activating STAT5, MAPK, and AKT compared with wild-type EpoR. Activation of STAT5, MAPK, and AKT was assessed at indicated Epo concentrations at 20 min postinduction by flow cytometry as described in B. D, Ba/F3 cells stably expressing K256R have reduced mitogenic activities compared with cells expressing wild-type EpoR. E, activation of MAPK and AKT is reduced in cells expressing K256R compared with wild-type EpoR. Error bars are standard deviations from at least three experiments.
Ubiquitination of EpoR

endosome to lysosome (15). Ubiquitination plays a crucial role in ligand-induced internalization and down-regulation of many cytokine receptors, despite differences in the molecular mechanisms used to couple ubiquitination to these trafficking processes. Like the EpoR, the prolactin and type I interferon (IFNAR1) receptors use ubiquitination to promote both receptor internalization and targeting to the lysosomes (12, 16). gp130 requires ubiquitination for lysosomal degradation, but not for ligand-induced internalization (14).

In addition to the contribution from EpoR ubiquitination shown in this study, p85 recruitment to cytoplasmic tyrosines Tyr429, Tyr431, and Tyr479 promotes EpoR internalization (3). Therefore, the EpoR employs multiple, and possibly cooperative and/or partially redundant, mechanisms to regulate receptor endocytosis. The relative contribution of these mechanisms to the overall EpoR endocytosis and whether one mechanism is preferred under certain physiological conditions are unclear. Interestingly, both ubiquitination-based and tyrosine-based mechanisms for EpoR internalization require JAK2 kinase activity, underscoring the contribution of JAK2 to EpoR signal transduction. Prolactin-stimulated ubiquitination and internalization of the prolactin receptor also requires JAK2 kinase activity (17); however, JAK2 activity is not a universal requirement for ubiquitination and internalization because internalization of the growth hormone receptor does not require JAK2 kinase activity (18), and the thrombopoietin receptor c-Mpl, while requiring JAK2 kinase activity for internalization, does not need JAK2 for ubiquitination (19, 20).

Our conclusions that ubiquitination of the EpoR is required for efficient ligand-induced receptor internalization and positively regulates downstream signaling by EpoR/JAK2 contrast published work of Meyer et al. (7), which suggested that EpoR ubiquitination is not important for internalization and lysosomal sorting. Instead, it affects Epo-induced receptor degradation and negatively regulates mitogenic activity. We reason that the different results regarding receptor internalization and sorting may stem from the differences in assay sensitivity, as degradation of internalized Epo was used to infer receptor internalization and sorting in that report. Alternatively, the human EpoR may behave differently from the murine EpoR. With regard to the role of EpoR ubiquitination in signaling, one possibility is that EpoR is ubiquitinated by multiple E3 ligases and that β-TrCP is only responsible for ubiquitination events that promote receptor internalization and degradation. Consistent with this possibility, Lys428, which is involved in lysosomal sorting/degradation of EpoR (Fig. 6), lies proximally to the consensus β-TrCP binding site. A second possibility is that β-TrCP ubiquitinates another component of the EpoR/JAK2 signaling complex, whose ubiquitination negatively regulates EpoR signaling.

Upon Epo stimulation, 5KR was capable of activating JAK2 normally; however, the amplitude of STAT5, ERK, and AKT activation was reduced. Consistent with these results, the mitogenic response of 5KR cells to Epo was defective compared with cells expressing wild-type EpoR. Therefore, JAK2 activation at the cell surface is not sufficient for optimal down-stream signaling of the EpoR. One hypothesis is that EpoR endocytosis kinetics and intracellular compartments critically contribute to sustaining and/or amplifying the STAT5, Ras/MAPK, and PI3K/AKT pathways. This hypothesis is based on recent findings regarding receptors such as the EGF receptor, TGFβ receptor, and G protein-coupled receptors, that intracellular compartments possess “signaling” capability to sustain signals originated from the cell surface as well as generate new signals (21, 22). For example, it was shown that ERK signaling may be enhanced in the presence of signaling components present in endosomes (23–25). Goh et al. (26) showed that continuous AKT activity requires EGF receptor internalization, and the endosomal adaptor protein WDFY2 was found to be required for maintaining insulin-stimulated AKT2 phosphorylation (27). In line with this hypothesis, 5KR and K256R, which are both defective in internalization, activate JAK2 but show attenuated activation of the ERK and AKT pathways. An alternative hypothesis is that ubiquitination of the EpoR may activate novel signaling pathways that contribute to mitogenic activity. A mutant mouse strain containing a severely truncated EpoR with no tyrosine residues in the cytoplasmic domain was viable with only slight alterations in erythropoiesis, contrary to the EpoR or JAK2 knock-out animals that die embryonically (28–31), indicating that novel non-tyrosine-based signaling pathways essential for erythropoiesis are yet to be identified. Interestingly, the severely truncated EpoR preserves three of five cytoplasmic lysines, including Lys256. Understanding the precise mechanisms underlying receptor endocytosis and signaling will provide insights into the regulation of these important molecules and diseases that are caused by excessive receptor signaling.

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REFERENCES

1. Constantinescu, S. N., Ghaffari, S., and Lodish, H. F. (1999) Trends Endocrinol. Metab. 10, 18–23
2. Richardson, T. D., Chohan, M., and Barber, D. L. (2005) Trends Cell Biol. 15, 146–155
3. Sulahian, R., Cleaver, O., and Huang, L. J. (2009) Blood 113, 5287–5297
4. Neumann, D., Wikström, L., Watowich, S. S., and Lodish, H. F. (1993) J. Biol. Chem. 268, 13639–13649
5. Walrafen, P., Verdier, F., Kadri, Z., Chrétien, S., Lacombe, C., and Mayeux, P. (2005) Blood 105, 600–608
6. Suzuki, N., Ohneda, O., Takahashi, S., Higuchi, M., Mukai, H. Y., Nakahata, T., Imagawa, S., and Yamamoto, M. (2002) Blood 100, 2279–2288
7. Meyer, L., Deau, B., Forejtmitová, H., Dumeníč, D., Margottin-Goguet, F., Lacombe, C., Mayeux, P., and Verdier, F. (2007) Blood 109, 5215–5222
8. Huang, L. J., Constantinescu, S. N., and Lodish, H. F. (2001) Mol. Cell Biol. 11, 1327–1338
9. Tong, W., Sulahian, R., Gross, A. W., Hendon, N., Lodish, H. F., and Huang, L. J. (2006) J. Biol. Chem. 281, 38930–38940
10. Yan, C. H., Yang, Y. C., Ruscetti, S. K., Kirken, R. A., Dai, R. M., and Li, C. C. (2000) J. Immunol. 165, 6372–6380
11. Wang, Y., Pennock, S., Chen, X., and Wang, Z. (2002) Mol. Cell Biol. 22, 7279–7290
12. Varghese, B., Barriere, H., Carbone, C. I., Banerjee, A., Swaminathan, G., Plotnikov, A., Xu, P., Peng, J., Goffin, V., Lukacs, G. L., and Fuchs, S. Y. (2008) Mol. Cell Biol. 28, 5275–5287
13. Barriere, H., Nemes, C., Du, K., and Lukacs, G. L. (2007) Mol. Biol. Cell
14. Tanaka, Y., Tanaka, N., Saeki, Y., Tanaka, K., Murakami, M., Hirano, T., Ishii, N., and Sugamura, K. (2008) Mol. Cell Biol. 28, 4805–4818
15. van Kerkhof, P., Alves dos Santos, C. M., Sachse, M., Klumperman, J., Bu, G., and Strous, G. I. (2001) Mol. Biol. Cell 12, 2556–2566
16. Kumar, K. G., Tang, W., Ravindranath, A. K., Clark, W. A., Croze, E., and Fuchs, S. Y. (2003) EMBO J. 22, 5480–5490
17. Swaminathan, G., Varghese, B., Thangavel, C., Carbone, C. J., Plotnikov, A., Kumar, K. G., Jablonski, E. M., Clevenger, C. V., Goffin, V., Deng, L., Frank, S. J., and Fuchs, S. Y. (2008) J. Endocrinol. 196, R1–7
18. Alves dos Santos, C. M., ten Broeke, T., and Strous, G. J. (2001) J. Biol. Chem. 276, 32635–32641
19. Saur, S. J., Sangkhiae, V., Geddis, A. E., Kaushansky, K., and Hitchcock, I. S. (2010) Blood 115, 1254–1263
20. Hitchcock, I. S., Chen, M. M., King, J. R., and Kaushansky, K. (2008) Blood 112(6), 2222–2231
21. Fehrenbacher, N., Bar-Sagi, D., and Philips, M. (2009) Mol. Oncol. 3, 297–307
22. Sorkin, A., and von Zastrow, M. (2009) Nat. Rev. Mol. Cell Biol. 10, 609–622
23. Kim, H. J., Taylor, L. J., and Bar-Sagi, D. (2007) Curr. Biol. 17, 455–461
24. Teis, D., and Huber, L. A. (2003) Cell Mol. Life Sci. 60, 2020–2033
25. Teis, D., Taub, N., Kurzbauer, R., Hilber, D., de Araujo, M. E., Erlacher, M., Offterdinger, M., Villunger, A., Geley, S., Bohn, G., Klein, C., Hess, M. W., and Huber, L. A. (2006) J. Cell Biol. 175, 861–868
26. Goh, L. K., Huang, F., Kim, W., Gygi, S., and Sorkin, A. (2010) J. Cell Biol. 189, 871–883
27. Walz, H. A., Shi, X., Chouinard, M., Bue, C. A., Navaroli, D. M., Hayakawa, A., Zhou, Q. L., Nadler, J., Leonard, D. M., and Corvera, S. (2010) J. Biol. Chem. 285, 14101–14108
28. Zang, H., Sato, K., Nakajima, H., McKay, C., Ney, P. A., and Ihle, J. N. (2001) EMBO J. 20, 3156–3166
29. Wu, H., Liu, X., Jaenisch, R., and Lodish, H. F. (1995) Cell 83, 59–67
30. Neubauer, H., Cumano, A., Müller, M., Wu, H., Huffstadt, U., and Pfeffer, K. (1998) Cell 93, 397–409
31. Parganas, E., Wang, D., Stravopodis, D., Topham, D. J., Marine, J. C., Teglund, S., Vanin, E. F., Bodner, S., Colamonici, O. R., van Deursen, J. M., Grossveld, G., and Ihle, J. N. (1998) Cell 93, 385–395