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Phosphorylation of rpS3 by Lyn increases translation of Multi-Drug Resistance (*MDR1*) gene

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Lyn, a tyrosine kinase that is activated by double-stranded DNA-damaging agents, is involved in various signaling pathways, such as proliferation, apoptosis, and DNA repair. Ribosomal protein S3 (RpS3) is involved in protein biosynthesis as a component of the ribosome complex and possesses endonuclease activity to repair damaged DNA. Herein, we demonstrated that rpS3 and Lyn interact with each other, and the phosphorylation of rpS3 by Lyn, causing ribosome heterogeneity, upregulates the translation of p-glycoprotein, which is a gene product of multidrug resistance gene 1. In addition, we found that two different regions of the rpS3 protein are associated with the SH1 and SH3 domains of Lyn. An *in vitro* immunocomplex kinase assay indicated that the rpS3 protein acts as a substrate for Lyn, which phosphorylates the Y167 residue of rpS3. Furthermore, by adding various kinase inhibitors, we confirmed that the phosphorylation status of rpS3 was regulated by both Lyn and doxorubicin, and the phosphorylation of rpS3 by Lyn increased drug resistance in cells by upregulating p-glycoprotein translation. [BMB Reports 2023; 56(5): 302-307]

INTRODUCTION

Ribosomal protein S3 is a protein component of the ribosome, which is a cellular structure that plays a central role in protein synthesis. It has extra-ribosomal functions, so called UV endonuclease III, to repair the DNA damage by UV irradiation (1-5). This enzyme cleaves UV irradiated DNA and AP (apyrimidic/apurinic) DNA, by cleaving the phosphodiester bond within a cyclobutane pyrimidine dimer and 3′ of AP site via a lyase mechanism (1, 5, 6). A previous study showed that rpS3 is secreted in cancer cell lines, and the level of secretion increased as the malignancy of the tumor cells increased (7, 8). RpS3 protein and mRNA expression levels are higher in both leukemia patients and leukemia cell lines than in their normal counterparts (8, 9). These results suggest that rpS3 can be used as an indicator of tumorigenesis. Therefore, in this study, we investigated the possible role of rpS3 as a key target of anti-cancer drug resistance.

In addition to its multiple extra-ribosomal functions, rpS3 possesses several post-translational modification motifs. Although many roles of these modifications are yet to be found, several different forms of modifications have been found both *in vitro* and *in vivo*, including phosphorylation, neddylation, sumoylation, and ubiquitination, thus imparting this protein versatile functions through ribosomal heterogeneity (8). Among these, rpS3 phosphorylation has been well-studied in conjunction with its functions of DNA repair and secretion (7, 8, 10-12).

RpS3 was also identified as a mammalian DNA repair endonuclease III, which has AP endonuclease/lyase activity and is involved in base excision repair (13). In addition, rpS3 is involved in apoptosis in cases of severe DNA damage in immune cells (12). These functions are important for maintaining cellular homeostasis and are regulated through several pathways. In our previous study, we found that rpS3 exists in the promyelocytic leukemia (PML) body within the nucleus and is modified by SUMO-1 (14). Hence, rpS3 may play a major role in B-cell growth and differentiation and be associated with acute myeloid leukemia along with PML. In this study, we examined the correlation between post-translational modifications and signaling systems of rpS3 that can integrate control pathways, such as protein synthesis, DNA damage recovery, and cell death.

The Lyn is a member of the Src family of tyrosine kinases and is mainly expressed in hematopoietic tissues (15, 16). It plays an important role in the regulating various cellular processes, such as cell growth, survival, migration, and immune response (17-20). This protein contains SH1 (kinase domain), SH2, and SH3 domains and a unique N-terminal sequence; the latter is dispensable for membrane localization via attachment of fatty acid myristate. The SH3 domain binds to proline-rich motifs, such as PXXP, and the SH2 domain binds to specific phosphotyrosine motifs. In addition, the SH2 and SH3 domains are implicated in substrate binding and the regulation of kinase activity via intramolecular interactions (18). Studies using cell fractionation and confocal microscopy have demonstrated that Lyn kinase also exists in the nucleus (19). Nuclear Lyn, but not other Src

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family tyrosine kinases, are activated after genotoxic treatment, such as 1-β-D-arabinofuranosylcytosine (Ara-C), ionizing irradiation, and adriamycin (20). Recently, BCR-ABL fusion proteins have been reported to activate Src tyrosine kinases, such as Lyn, Hck, and Fgr, to induce leukemia (21). Lyn has been studied mainly in the growth and differentiation processes of B lymphocytes (22); however, many studies have been recently conducted on its activation caused by DNA damage (23). Studies on protein synthesis have revealed various regulatory mechanisms of ribosomal S6 kinase, and some studies on regulatory mechanisms for other ribosomal proteins have also been revealed (24).

P-glycoprotein (p-gp) or multidrug resistance protein (MDR1) is a type of protein that is expressed in the membranes of certain cells, including cells in the blood-brain barrier and the cells lining the small intestine. It is a member of the ATP-binding cassette (ABC) family of transporters and is responsible for pumping drugs and other xenobiotics out of cells, making them less effective. ATP-binding cassette (ABC) transporters are known to have multiple drug binding sites, which allow them to transport a diverse range of substrates, including drugs such as doxorubicin (25). High expression levels of ABCB1 (also known as P-glycoprotein or P-gp) are commonly associated with drug resistance in cancer cells (26), both acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL) (27).

The multidrug resistance protein 1 (MDR1) is involved in the extrusion of a wide variety of drugs and xenobiotics from cells, leading to decreased intracellular drug accumulation and, ultimately, reduced drug efficacy (28). MDR1 has also been implicated in resistance to certain anticancer, anti-inflammatory, and antiviral drugs, among others (26). P-gp is known to be regulated by post-translational modifications such as phosphorylation and N-glycosylation (29).

Doxorubicin (DOX) is a chemotherapy drug that works by inhibiting DNA replication and inducing DNA damage in cancer cells. However, the effects of doxorubicin are often limited by overexpression of P-glycoprotein (P-gp) (30). DOX is widely used and highly effective for the treatment of breast cancer, sarcoma, leukemia, and lymphoma (31). Inhibition of p-gp as a method to reverse MDR in patients with cancer has been extensively reviewed; however, the results have generally been disconcerting (32).

Acute PML and chronic myeloid leukemia are associated with the activation of Src tyrosine kinases; thus, investigating the modification of the rpS3 protein could provide important insights into cancer treatment.

Herein, we found that rpS3 interacts with Lyn and that this interaction elicits the phosphorylation of rpS3. Furthermore, we investigated the effect of Lyn on rpS3 phosphorylation by adding various kinase inhibitors. We also demonstrated that the phosphorylation of rpS3 by Lyn regulates p-gp translation.

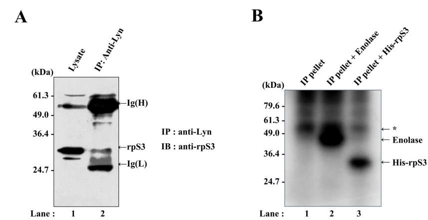

Fig. 1. RpS3 interacts with Lyn *in vitro*. (A) HEK293T cells were transfected with pEGFPc1 or pEGFPc1-Lyn plasmids. Cell extracts were immunoprecipitated (IP) using anti-Lyn antibody, and immunprecipitated proteins were resolved by 10% SDS-PAGE and immunoblotted (IB) using the indicated antibody. Ig(H); heavy chain, Ig(L); light chain. (B) Lyn interacts with and phosphorylates rpS3. MPC-11 cell lysates were immunoprecipitated with Lyn antibody, and an *in vitro* kinase assay was performed using purified His-rpS3. Proteins were analyzed by 10% SDS-PAGE and subsequent autoradiography. Enolase was used as a positive control. Asterisk indicates Lyn autophosphorylation. Phosphorylation was detected by isotope labeling with γ-^{32}P ATP followed by phosphor imaging analysis. The data represent three independent experiments.

RESULTS

RpS3 is phosphorylated after the interaction with Lyn

Our previous studies using a yeast two-hybrid system revealed that Lyn is a putative binding partner of rpS3 (14). To demonstrate the interaction of the tyrosine kinase Lyn with rpS3, we performed a co-immunoprecipitation assay using overexpression of pEGFPc1-Lyn. HEK293T cells were transfected with either a blank GFP vector or GFP vector carrying Lyn protein. As shown in Fig. 1A, the interaction between rpS3 and GFP-Lyn was verified (lane 2); however, the interaction between rpS3 and lysate was not verified (lane 1). Next, we investigated whether this interaction occurs between endogenous Lyn and rpS3. We confirmed specific interactions between endogenous proteins in MPC-11 cells. Conversely, endogenous rpS3 was precipitated with antibodies against endogenous Lyn. These results indicated that rpS3 has a strong physical interaction with Lyn *in vitro* (Fig. 1A).

As Lyn is a well-known protein tyrosine kinase, we tested the possibility of rpS3 phosphorylation by Lyn. For this purpose, we performed an immunocomplex kinase assay with an anti-Lyn antibody, and recombinant His-tagged rpS3 protein was used as a substrate. Immunoprecipitates were incubated with acid-treated enolase as a positive control. As shown in Fig. 1B, autophosphorylation of Lyn demonstrated that rpS3 protein inhibits the kinase activity of Lyn. As expected, recombinant His-tagged rpS3 protein was phosphorylated by Lyn. Collectively, these results suggest that rpS3 protein (lane 3) can be phosphorylated *in vitro* as much as enolase (lane 2), which is the positive substrate of Lyn and acts as a regulator of Lyn.

RpS3 interacts with SH3 domain of Lyn

To determine the region that is crucial for the interaction of

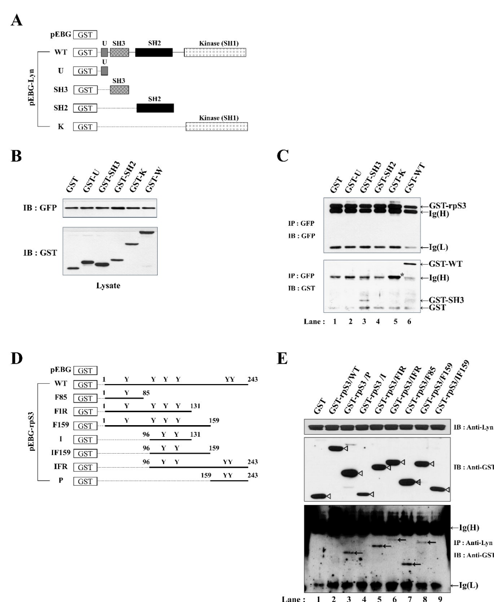

Fig. 2. Identification of interaction domain of rpS3 with Lyn. (A) Schematic representation of GST-Lyn deletion constructs. (B) HEK293T cells were cotransfected with pEBG-Lyn deletion mutants and pEGFP- rpS3. Immunoblotting assay of the different GST-Lyn deletion mutants. GST and GST-Lyn fusion proteins (WT or deletion mutant, lower panel). Bacterial lysates containing each protein were prepared as described in Materials and Methods and incubated with glutathione-agarose. The glutathione agarose was then washed, and bound proteins were eluted with 20 mM free glutathione. The purified GST (lane 1), GST-Lyn unique domain (lane 2), GST-Lyn SH3 (lane 3), GST-Lyn SH2 (lane 4), GST-Lyn kinase domain (lane 5), and GST-Lyn wild type (lane 6) fusion proteins were resolved by electrophoresis on an 10% SDS-PAGE and visualized by immunoblotting assay with GFP and GST antibodies. (C) Lyn domain interacted with rpS3 using by immunoprecipitation (upper panel) and co-immunoprecipitation (lower panel) assay. Cell extracts were immunoprecipitated (IP) using anti-GFP antibody, and immunprecipitated proteins were resolved by 10% SDS-PAGE and immunoblotted (IB) using the indicated antibody. Ig(H), heavy chain, Ig(L), light chain. Asterisks indicate GST-K domain (lower panel, lane 5). (D) Schematic representation of GST-rpS3 deletion constructs. The numbers indicate amino acid (a.a) positions in rpS3. (E) HEK293T cells were cotransfected with pEBG-rpS3 deletion mutants and pcDNA3-Lyn. After 24 h, cells were lysed, and extracts were pulled down with glutathione-Sepharose beads. Isolated GST fusion proteins were resolved by 10% SDS-PAGE and immunoblotted with Lyn (upper panel) and GST (middle panel) antibodies. Open arrowheads indicate the positions of GST and GST-rpS3 constructs and GST, respectively. WT, wild type. Y, tyrosine. RpS3 deletion mutants interacted with Lyn by immunoprecipitation assay (lower panel). Cell extracts were immunoprecipitated (IP) using anti-Lyn antibody, and immunprecipitated proteins were resolved by 10% SDS-PAGE and immunoblotted (IB) using the indicated antibody. Ig(H), heavy chain, Ig(L), light chain. Open arrowheads indicate the interaction positions of pEBG-rpS3 deletion mutants with pcDNA3-Lyn, respectively. The data represent three independent experiments.

Lyn with rpS3, a fine deletion analysis of the N- and C-termini was performed. For this purpose, we generated GST-tagged Lyn deletion constructs, as described in Fig. 2A. All the GST-tagged constructs were transferred into HEK293T cells, and GST pull-down assays were performed to target rpS3 (Fig. 2B). Furthermore, GST-tagged Lyn deletion mutants were co-transfected with a plasmid bearing GFP-tagged rpS3 into HEK293T cells, and an *in vitro* binding assay was performed (Fig. 2C). These results demonstrate that rpS3 binds to the SH3 domain (63-123, lane 3) and SH1 domain of Lyn (240-500, lane 5) but not to the unique N-terminal domain (1-62, lane 2) and SH2 domain (128-226, lane 4). As the binding affinity of the SH1 domain was stronger than that of SH3, we suggest that the SH1 domain of Lyn is the major binding domain with rpS3 (Fig. 2C). Furthermore, to examine the role of the SH3 domain of Lyn, we co-transfected the GFP-tagged SH3 domain of Lyn and rpS3 into Cos-1 cells (Supplementary Fig. 1). Although the wild type Lyn interacted very weakly, the SH3 domain of Lyn strongly interacted with rpS3 (lanes 3 and 4).

The RpS3 protein is highly basic and contains two domains: the KH domain and the S3-C terminal domain. We tested which region of rpS3 interacted with the Lyn, N-, and C-terminal deletion constructs, as described in Fig. 2D (33). Each deletion mutant was co-expressed with Lyn in HEK293T cells, and immunoprecipitation analysis was performed using cell lysates. Interestingly, rpS3 appeared to interact with Lyn via both N-terminal and C-terminal domains (Fig. 2E, lanes 5, 6, 7, 9; Open arrowheads). This result implies that there are more than one binding sites in the rpS3. More specifically, the Lyn-binding region of rpS3 lies in the N-terminal (rpS3: 1-85, lane 7) and C-terminal regions (rpS3: 159-242, lane 3). Overall, these results suggest that the association between Lyn and rpS3 occurs by direct interaction of the Lyn SH3 domain with the C-terminal region of rpS3 (159-242, lane 3), or it may occur by the interaction of the Lyn SH1 domain with the N-terminal region of rpS3 (1-85, lane 7). The binding activity of rpS3-P (159-242, Fig. 2E, lane 3; GST-rpS3 C-terminal, Supplementary Fig. 2, lane 9) was equal to that of the wild type. These results demonstrated that the binding region of rpS3 for Lyn is within the C-terminus of the rpS3 protein. The C-terminal region (Fig. 2E, lane 3 and Supplementary Fig. 2, lane 9) contains three putative proline-rich motifs, such as PxxP, that can bind to the SH3 domain of Lyn, which raises the possibility that the SH3 domain of Lyn would take part in the interaction.

Tyrosine 167 of rpS3 is a critical residue for the phosphorylation by Lyn *in vitro*

Human rpS3 has several phosphorylation target sites, mostly serine/threonine residues (31, 32). To identify the tyrosine residue phosphorylated by Lyn, we generated a series of rpS3 mutations in which each tyrosine was substituted with phenylalanine (Fig. 3A). Subsequently, an immunocomplex kinase assay was performed (Fig. 3B). Substitution of all six tyrosine residues with phenylalanine (KS2, lane 4) abrogated Lyn-mediated phosphorylation. Interestingly, the double substitution mutant (KS6, Lane 8) also abrogated phosphorylation. Based on these results, Y166 and Y167 were assumed to be important for phosphory-

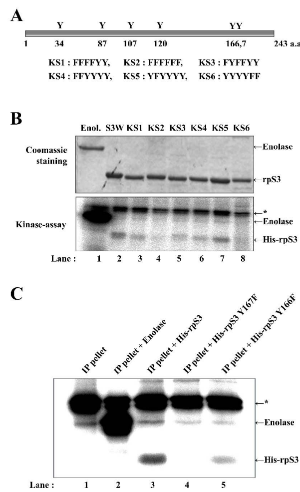

Fig. 3. RpS3 phosphorylates Lyn at Y167. (A) Schematic representation of GST-rpS3 point mutation (Tyr→Phe) constructs. The numbers indicate tyrosine positions in rpS3. (B) MPC-11 cell lysates were immunoprecipitated with Lyn antibody, and an *in vitro* kinase assay was performed using purified pET21a-rpS3. Proteins were analyzed by 10% SDS-PAGE and subsequent Coomassie blue staining (upper panel) and autoradiography (lower panel). Enolase was used as a positive control. Asterisks indicate Lyn autophosphorylation. (C) MPC-11 cell lysates were immunoprecipitated with Lyn antibody, and an *in vitro* kinase assay was performed using purified His-rpS3 Y166 and Y167. Proteins were analyzed by 10% SDS-PAGE and subsequent autoradiography. Enolase was used as a positive control. Asterisk indicates Lyn autophosphorylation. The data represent three independent experiments.

lation at rpS3. We generated appropriate single substitution mutants (Fig. 3C) for further investigation. The results of this analysis revealed that Y166 was weakly phosphorylated, while the Y167 residue was not phosphorylated. Therefore, we concluded that Lyn-induced rpS3 phosphorylation was eliminated by Y167F mutation of rpS3 and confirmed that the Y167 residue of rpS3 is the critical phosphorylation site for Lyn.

We tested the effects of DOX, an anti-cancer drug that inhibits topoisomerase II, on the association of Lyn with rpS3. When GFP-rpS3 was treated with DOX, the interaction between rpS3 and Lyn increased (Supplementary Fig. 3, lower panel, lane 4). The results of this study suggested that DOX plays an important role in the interaction between rpS3 and Lyn.

Lyn may be activated by genotoxic agents, such as Ara-C (1-beta-D-Arabinofuranosylcytosine), adriamycin (DOX), and ionizing radiation (23). Therefore, we investigated whether the phosphorylation of rpS3 increases after treatment with genotoxins. As expected, the higher the concentration of Ara-C, the higher was the level of phosphorylated rpS3 (Supplementary Fig. 4, lane 3). However, the result may not be owing to a specific phosphorylation by Lyn; hence, we subsequently studied the effect of a global and specific inhibitor of Src family tyrosine kinases such as PP2 and genistein. Overall, rpS3 phosphorylation decreased in a dose-dependent manner. Thus, these results suggest that the phosphorylation of rpS3 is due to Src tyrosine family kinases, such as Lyn.

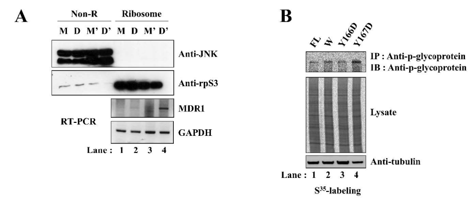

Fig. 4. Identification of activity translated *MDR1* mRNA. (A) Non-ribosomal and ribosomal protein were fractionated. Both fractions were immunoblotted with JNK and rpS3 antibodies. *MDR1* and *GAPDH* transcripts from the cells were amplified by RT-PCR and detected by Southern blot hybridization using oligonucleotide probes. GAPDH was used as an internal control. (B) S^{35}-Methione labeling was used to investigate *in vivo* quantitative measurements of p-glycoprotein synthesis of Y166D and Y167D (a phosphorylation-mimetic mutant) in MPC11-DOX cells. Equal amounts of proteins were resolved by 10% SDS-PAGE and analyzed by autoradiography. Control FL (Flag), wild type rpS3 (W) and Y166D, Y167D; tyrosine to aspartic acid substituted mutants. Tubulin antibody was used as an internal control. The data represent three independent experiments.

Increase of *MDR1* expression in DOX-resistant cells

The levels of *MDR1* mRNA and p-gp in the DOX-resistant MPC-11 cell line (MPC11-DOX) were significantly higher than that in the MPC-11 parent cells (Supplementary Fig. 5A). In addition, the level of tyrosine phosphorylation of rpS3 in MPC11-DOX cells increased significantly compared to that in MPC-11 parent cells (Supplementary Fig. 5B, C, lane 6). These results confirmed that phosphorylation of the tyrosine residue of rpS3 was increased in MPC11-DOX cells. DOX induction of p-gp expression occurred owing to the phosphorylated status of rpS3. Although a DOX-resistant cell line was established, there was little change in the ribosome content of this cell line through the ribosomal profile, particularly in polysomes (Supplementary Fig. 5D). We examined the translation levels of c-Jun NH2-Terminal kinase and rpS3 proteins in non-ribosome and ribosome fractions (34) and compared the levels of *MDR1* mRNA in parent cells (M, M′) and MDR11-DOX cells (D, D′) using reverse transcription polymerase chain reaction (Fig. 4A). The results demonstrated that in the ribosomal protein fractions, MDR1 mRNA was higher in MPC11-DOX cells (D, Fig. 4A, lane 2) than in parental cells (M, Fig. 4A, lane 1). MPC11-DOX cells with enhanced resistance to DOX (D′, Fig 4A, lane 4) showed a marked increase in *MDR1* mRNA compared to the original MPC11-DOX cells (D, Fig. 4A, lane 2). Altogether, these results suggest that selection for drug resistance, by long-term exposure to drugs, led to the establishment of a boosted DOX-resistant cell line D′, in which the translational block of *MDR1* mRNA was overcome such that the mRNA was translated and p-gp was overexpressed (35). Based on these results (Fig. 3C), we performed *in vivo* quantitative measurements of

p-gp synthesis following control rpS3-WT or Y166D and Y167D as substrates (phosphomimetic form) in MPC11-DOX cells using [S^{35}]-methionine labeling (Fig. 4B). P-gp of Y166D (lane 3) was similar to that of the wild type. In the case of Y167D (lane 4), p-gp expression level was three-fold higher than that of the wild type (lane 2). This suggests that protein synthesis (i.e., translation) of p-gp is upregulated by the phosphorylation of rpS3 by Lyn.

DISCUSSION

RpS3 is an essential constituent of the 40S subunit of the ribosome and is involved in protein translation. However, the extraribosomal functions of many ribosomal proteins have not yet been elucidated. RpS3 has been extensively studied for its functions outside of the translational machinery, and multiple roles in DNA damage repair and apoptosis have been reported (1, 8, 12).

Overexpression of rpS3 in colorectal cancers has been reported, and the relationship between rpS3 and cancer development has long been suspected. In this regard, we studied the relationship between leukemia-related carcinogenesis and the modification of rpS3. Based on the regulatory properties of Lyn in tumorigenesis and its biochemical relationships with various binding partners, we hypothesized that the relationship between rpS3 and Lyn could be functionally involved in the drug resistance of MPC11 cells. The antitumor effect of DOX primarily involves topoisomerase-mediated double-strand DNA breaks with the subsequent triggering of DNA damage-associated cell cycle arrest and apoptosis pathways. In this study, we showed the interaction between the rpS3 protein and Lyn, demonstrating the phosphorylation of rpS3 by Lyn (Fig. 1). The SH3 domain of Lyn contains a module that can bind to the proline-rich motif (PxxP) (25-27). Although three putative proline-rich motifs that lie in the C-terminus of rpS3 are not exactly matched with the conserved proline-rich motifs, our results showed that the putative proline-rich motifs of rpS3 interacted with the SH3 domain of Lyn (Fig. 2C). However, to identify the binding sites between the rpS3 and SH3 domains of Lyn, further studies need to be carried out, such as point mutations in three putative proline-rich motifs. Furthermore, we showed that the SH1 domain of Lyn interacted with rpS3 (Fig. 2C).

The results of kinase assay also revealed that rpS3 is directly phosphorylated by Lyn, and the level of phosphorylation is comparable to that for enolase, a typical phosphorylation target of Lyn (Fig. 1B). In addition, we demonstrated that rpS3 inhibits the autophosphorylation of Lyn. Collectively, our data demonstrated that rpS3 is a substrate of Lyn kinase. Lyn phosphorylates 166Y and 167Y in rpS3 (Fig. 3C), and Y167 is a critical residue for rpS3 function.

Lyn is activated by various agents that arrest DNA replication or damage (20, 34). Herein, we demonstrated that the phosphorylation of rpS3 was increased by treatment with genotoxic agents and decreased by the addition of a specific inhibitor of Src family tyrosine kinases, such as PP2 (Supplementary Fig. 4). In addition, we showed that phosphorylation was completely abolished by the addition of general tyrosine kinase inhibitors, such as genistein.

Although further studies are needed to clarify the precise role of Lyn phosphorylation on rpS3, we suggest that phosphorylation may affect the repair activity of rpS3.

RpS3 is subjected to various post-translational modifications, such as phosphorylation, sumoylation, methylation, and ubiquitination (8, 11, 13, 33, 36, 37). These modifications also lead to ribosome heterogeneity, resulting in differential translational products (38).

Various modifications and changes in the expression level of the rpS3 protein occur when cells are under various types of stress. We confirmed that phosphorylation of the tyrosine residue of rpS3 was increased in response to genotoxic stress, and p-gp, with the phospho-mimic form of Y167D, was higher than that of the wild type (Fig. 4B). This suggests that p-gp protein synthesis is regulated by the phosphorylation of rpS3 by Lyn via ribosome heterogeneity.

In conclusion, our study identified the interaction between Lyn and rpS3 and the phosphorylation of rpS3 by activated Lyn. This phosphorylation of rpS3 by Lyn increased the translation of p-gp, a gene product of *MDR1* (multi-drug resistance gene 1). This phosphorylation of rpS3 by Lyn may result in increased drug resistance in cancer cells.

MATERIALS AND METHODS

Further detailed information is provided in the Supplementary Information.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

REFERENCES

1. Kim J, Chubatsu LS, Admon A, Stahl J, Fellous R and Linn S (1995) Implication of mammalian ribosomal protein S3 in the processing of DNA damage. J Biol Chem 270, 13620-13629
2. Yacoub A, Augeri L, Kelley MR, Doetsch PW and Deutsch WA (1996) A Drosophila ribosomal protein contains 8-oxoguanine and abasic site DNA repair activities. EMBO J 15, 2306-2312
3. Jung SO, Lee JY and Kim J (2001) Yeast ribosomal protein S3 has an endonuclease activity on AP DNA. Mol Cells 12, 84-90
4. Lee CH, Kim SH, Choi JI, Choi JY, Lee CE and Kim J (2002) Electron paramagnetic resonance study reveals a putative iron-sulfur cluster in human rpS3 protein. Mol Cells 13, 154-156

5. Kanno S, Iwai S, Takao M and Yasui A (1999) Repair of apurinic/apyrimidinic sites by UV damage endonuclease; a repair protein for UV and oxidative damage. Nucleic Acids Res 27, 3096-3103
6. Hegde V, Kelley MR, Xu Y, Mian IS and Deutsch WA (2001) Conversion of the bifunctional 8-oxoguanine/beta-delta apurinic/apyrimidinic DNA repair activities of Drosophila ribosomal protein S3 into the human S3 monofunctional beta-elimination catalyst through a single amino acid change. J Biol Chem 276, 27591-27596
7. Kim Y, Kim HD, Youn B, Park YG and Kim J (2013) Ribosomal protein S3 is secreted as a homodimer in cancer cells. Biochem Biophys Res Commun 441, 805-808
8. Kim Y, Lee MS, Kim HD and Kim J (2016) Ribosomal protein S3 (rpS3) secreted from various cancer cells is N-linked glycosylated. Oncotarget 7, 80350-80362
9. Hua W, Yue L and Dingbo S (2016) Over-expression of rps3 promotes acute lymphoblastic leukemia growth and progress by down-regulating COX-2 through NF-κb pathway. Blood 128, 3927
10. Kim SH, Lee JY and Kim J (2005) Characterization of a wide range base-damage-endonuclease activity of mammalian rpS3. Biochem Biophys Res Commun 328, 962-967
11. Kim TS, Kim HD and Kim J (2009) PKCdelta-dependent functional switch of rpS3 between translation and DNA repair. Biochim Biophys Acta 1793, 395-405
12. Jang CY, Lee JY and Kim J (2004) RpS3, a DNA repair endonuclease and ribosomal protein, is involved in apoptosis. FEBS Lett 560, 81-85
13. Park YJ, Kim SH, Kim TS et al (2021) Ribosomal protein S3 associates with the TFIIH complex and positively regulates nucleotide excision repair. Cell Mol Life Sci 78, 3591-3606
14. Jang CY, Shin HS, Kim HD, Kim JW, Choi SY and Kim J (2011) Ribosomal protein S3 is stabilized by sumoylation. Biochem Biophys Res Commun 414, 523-527
15. Yi TL, Bolen JB and Ihle JN (1991) Hematopoietic cells express two forms of lyn kinase differing by 21 amino acids in the amino terminus. Mol Cell Biol 11, 2391-2398
16. Yamanashi Y, Kakiuchi T, Mizuguchi J, Yamamoto T and Toyoshima K (1991) Association of B cell antigen receptor with protein tyrosine kinase Lyn. Science 251, 192-194
17. Suzuki T, Shoji S, Yamamoto K et al (1998) Essential roles of Lyn in fibronectin-mediated filamentous actin assembly and cell motility in mast cells. J Immunol 161, 3694-3701
18. Grishin A, Sinha S, Roginskaya V et al (2000) Involvement of Shc and Cbl-PI 3-kinase in Lyn-dependent proliferative signaling pathways for G-CSF. Oncogene 19, 97-105
19. Corey SJ, Dombrosky-Ferlan PM, Zuo S et al (1998) Requirement of Src kinase Lyn for induction of DNA synthesis by granulocyte colony-stimulating factor. J Biol Chem 273, 3230-3235
20. Mayer BJ (2001) SH3 domains: complexity in moderation. J Cell Sci 114, 1253-1263
21. Kharbanda S, Saleem A, Yuan ZM et al (1996) Nuclear signaling induced by ionizing radiation involves colocalization of the activated p56/p53lyn tyrosine kinase with p34cdc2. Cancer Res 56, 3617-3621
22. DeFranco AL, Chan VW and Lowell CA (1998) Positive and negative roles of the tyrosine kinase Lyn in B cell function. Semin Immunol 10, 299-307
23. Yoshida K, Weichselbaum R, Kharbanda S and Kufe D (2000) Role for Lyn tyrosine kinase as a regulator of stress-activated protein kinase activity in response to DNA damage. Mol Cell Biol 20, 5370-5380
24. Hu Y, Liu Y, Pelletier S et al (2004) Requirement of Src kinases Lyn, Hck and Fgr for BCR-ABL1-induced B-lymphoblastic leukemia but not chronic myeloid leukemia. Nat Genet 36, 453-461
25. Satake K, Tsukamoto M, Mitani Y et al (2015) Human ABCB1 confers cells resistance to cytotoxic guanidine alkaloids from Pterogyne nitens. Biomed Mater Eng 25, 249-256
26. Fojo AT, Ueda K, Slamon DJ, Poplack DG, Gottesman MM and Pastan I (1987) Expression of a multidrug-resistance gene in human tumors and tissues. Proc Natl Acad Sci U S A 84, 265-269
27. Baudis M, Prima V, Tung YH and Hunger SP (2006) ABCB1 over-expression and drug-efflux in acute lymphoblastic leukemia cell lines with t(17;19) and E2A-HLF expression. Pediatr Blood Cancer 47, 757-764
28. Arceci RJ, Baas F, Raponi R, Horwitz SB, Housman D and Croop JM (1990) Multidrug resistance gene expression is controlled by steroid hormones in the secretory epithelium of the uterus. Mol Reprod Dev 25, 101-109
29. Lelong-Rebel IH and Cardarelli CO (2005) Differential phosphorylation patterns of P-glycoprotein reconstituted into a proteoliposome system: insight into additional unconventional phosphorylation sites. Anticancer Res 25, 3925-3935
30. Kuo MT (2009) Redox regulation of multidrug resistance in cancer chemotherapy: molecular mechanisms and therapeutic opportunities. Antioxid Redox Signal 11, 99-133
31. Meredith AM and Dass CR (2016) Increasing role of the cancer chemotherapeutic doxorubicin in cellular metabolism. J Pharm Pharmacol 68, 729-741
32. Thomas H and Coley HM (2003) Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein. Cancer Control 10, 159-165
33. Kim TS, Jang CY, Kim HD, Lee JY, Ahn BY and Kim J (2006) Interaction of Hsp90 with ribosomal proteins protects from ubiquitination and proteasome-dependent degradation. Mol Biol Cell 17, 824-833
34. Kim TS, Kim HD, Park YJ et al (2019) JNK activation induced by ribotoxic stress is initiated from 80S monosomes but not polysomes. BMB Rep 52, 502-507
35. Beausoleil SA, Villen J, Gerber SA, Rush J and Gygi SP (2006) A probability-based approach for high-throughput protein phosphorylation analysis and site localization. Nat Biotechnol 24, 1285-1292
36. Shin HS, Jang CY, Kim HD, Kim TS, Kim S and Kim J (2009) Arginine methylation of ribosomal protein S3 affects ribosome assembly. Biochem Biophys Res Commun 385, 273-278
37. Jung Y, Kim HD, Yang HW, Kim HJ, Jang CY and Kim J (2017) Modulating cellular balance of Rps3 mono-ubiquitination by both Hel2 E3 ligase and Ubp3 deubiquitinase regulates protein quality control. Exp Mol Med 49, e390
38. Yang HW, Kim HD, Kim TS and Kim J (2019) Senescent cells differentially translate senescence-related mRNAs via ribosome heterogeneity. J Gerontol A Biol Sci Med Sci 74, 1015-1024