약학석사학위논문

Functional Implications of Leucyl-tRNA Synthetase in Colorectal Cancer

대장암 유발에 관여하는 Leucyl−tRNA synthetase의 기능적 의의

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이 논문을 약학석사학위논문으로 제출함

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2015 년 12 월

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ABSTRACT

Colorectal cancer is one of challenging cancers that has high incidence rate and death rate at the same time [1]. However, since the effective targets and chemotherapeutic agents used in clinic are lack, it is necessary to identify the new drug target for controlling colorectal cancer.

Here we found that leucyl-tRNA synthetase (LRS), known as a leucine sensor of mTOR signaling, is highly expressed in colorectal cancer patient tissues as well as diverse colorectal cancer cell lines [2, 3]. LRS expression significantly promotes cancer cell growth and proliferation as determined by immunoblotting, immunohistochemistry, [^{35}S] Met incorporation, anchorage independent growth, and xenograft assay. In addition, LRS expression is positively correlated with phosphorylation of S6Kinase which is a downstream effector of mTOR. Silencing of LRS attenuates the phosphorylation of S6K, oncogenic growth, tumor mass while increase of LRS expression enhances tumorigenic propensity. Therefore, the regulation of mTOR-S6kinase pathway via suppression of LRS expression is effective way to control cancer cell growth. Taken together, this is consistent with observation that LRS may be suggested as a potential therapeutic target to control colorectal cancer and that effective tools for LRS are needed further validation and study.

Key words : Leucyl-tRNA synthetase, mTOR, colorectal cancer, tumorigenicity, therapeutic target

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INTRODUCTION

Aminoacyl-tRNA synthetases (ARSs) are known as essential enzymes linking the information from DNA to proteins [4]. ARSs also present their essential role in maintenance of cell viability, showing well conserved catalytic domain in the eukaryotes as well as in prokaryotes [5]. They have other domains which make the functional versatility through assembling with ARSs each other or with other parts of cellular factors. This structural diversity also leads to their functional diversity [6, 7]. According to numerous studies, ARSs play a role in various signaling pathways related to human diseases such as cancer [8, 9]. Various ARSs are also linked to signaling pathways affecting tumorigenesis [7]. In fact, many cancer types show the aberrant expression and mutation of ARSs in themselves.

Among 20 types of ARSs, LRS is catalytically in charge of transferring leucine to leucyl-tRNA during the initial step of protein synthesis. LRS also has a number of non-canonical functions other than its classical function [10-12]. In previous study, it was reported that LRS has a function as a mediator of the mTOR pathway [2]. mTOR pathway is widely acknowledged as one of the key pathways that are involved in human cancer [13, 14]. It is particularly known that mTORC1 pathway is deeply involved in cell growth through the regulation of downstream effectors [14, 15]. Meanwhile, colorectal cancer has the fourth highest incidence rate followed by breast, prostate and lung cancer. Colorectal cancer is

ranked as the fourth highest mortality rate of all cancer types [1]. Taken together, colorectal cancer is one of the most challenging cancers showing high incidence rate and death rate at the same time. According to previous reports, mTOR and mTOR related proteins were highly expressed in colorectal cancer and mTOR pathway is hyperactivated as well [3]. Based on these evidences, we started to study about correlation between LRS and mTOR signaling pathway and its influence on tumor formation through its oncogenic phenotypes.

MATERIALS AND METHODS

Materials

Primary antibodies we used were LRS (Neomics, Cat. NMS-01-0007), phospho-S6(S240/244) (Cell signaling, #2215), Total S6 (Cell signaling, #2217), phospho-S6K(T389) (Cell signaling, #9206), Total S6K (Cell signaling, #9202), Actin (Sigma aldrich, Cat. A2228), phospho-AKT(S473) (cell signaling, #4060), phospho-AKT(T308) (Cell signaling, #4056), Total AKT (Cell signaling, #9272), phospho-ERK1/2 (cell signaling, #9101), total ERK (cell signaling, #5013), Myc (Santa cruz, sc-40), EPRS (Neomics, Cat. NMS-01-0004), MRS (Neomics, Cat. NMS-01-0003), Total mTOR (Cell signaling, #2972) and Ki67 (Santa cruz, sc-15402). Secondary antibodies we used were Rabbit (Thermo scientific Immunopure Ab, Host Goat-Anti, Antigen Rabbit IgG(H+L), Cat. 31460), and Mouse (Thermo scientific Immunopure Ab, Host Goat-Anti, Antigen Mouse IgG(H+L), Cat. 31430).

Cell culture

HEK293T cells were cultured in High glucose Dulbecco's Modified Eagle's Medium (DMEM) (with 400 mg/L Glutamine, 4500 mg/L Glucose, Sodium pyruvate, Hyclone, Cat. 30243.01) with 10% fetal bovine serum (FBS, Hyclone, Cat. SH30919.03) and 100 μg/mL

penicillin and streptomycine (10000 units/mL penicillin/10000 μg/mL streptomycine, Hyclone, Cat. SV30010) at 37℃ in 5% CO_2 incubator.

SW620 inducing myc-LRS and LRS shRNA cells were cultured in RPMI-1640 medium (with 24 mM HEPES and L-Glutamine, Hyclone, Cat. SH30255.01) with 10% fetal bovine serum, 100 μg/mL penicillin and streptomycine at 37℃ in 5% CO_2 incubator. And 1 μg/mL doxycycline is added to cultured cell line 2 days before the experiment.

Immunohistochemistry

The tumor mass was removed from transgenic mouse and the tumor was washed with PBS, fixed in 10% paraformaldehyde at 4℃ overnight. After washing with tap water, the tumor was embedded in paraffin. The paraffin block was sliced as stepwise section slides. Then, the antibodies were applied on rehydrated tissue sections which pretreated with Target Retrieval Solution for staining. An EnVision system (HRP/DAB) (DAKO) was used for visualization.

Antibodies were obtained from the following sources: antibodies to LRS and EPRS were from Neomics (Cat. NMS-01-0007, NMS-01-0004). phospho-S6K(T389) and phospho-S6 (S240/244) were obtained from Cell Signaling Technology (Cat.#9206, #2215). Antibody to myc (9E10) and Ki67 were obtained from Santacruz (Cat. sc-40, sc-15402).

Immunoblotting

Cells were lysed in cold lysis buffer (50 mM Tris (pH 7.4), 0.5% Triton X-100, 0.5% NP-40, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, supplemented with protease and phosphatase inhibitors) for 30 minutes at 4℃. Then, each lysate was collected into eppendorf tubes and centrifuged at 13,200 rpm for 15 minutes at 4℃. The supernatant proteins were quantified by Bradford assay (BioRad, Cat. 500-0006). 5X sample buffer and lysis buffer were added to quantified proteins to make the final samples. After boiling samples for 5 minutes, samples were loaded on SDS page gels and separated by electrophoresis. The gels which obtained the proteins were transferred to PVDF (polyvinylidenefluoride) membranes (Milipore, Cat. IPVH 00010) at 1.3 mA, 25 mV for 15 minutes. The membranes were incubated with 10% skim milk solution based 0.5% TBS-T for 30 minutes to prevent non-specific bindings. After removing the skim milk solution, primary antibodies were added to the each membrane during overnight at 4℃. Next day, the membranes were washed with 0.5% TBS-T buffer for 5 minutes, 3 times repeatedly. Secondary antibodies were added for 1 hour 30 minutes. Membrane was washed equally to the previous step, and ECL solution (Santacruz, cat. sc-2048, GE Healthcare, cat. RPN2232) was applied to the membrane.

Antibodies were obtained from the following sources: antibodies to LRS, EPRS and MRS were obtained from Neomics (Cat. NMS-01-0007, NMS-01-0004, NMS-01-0003). Antibodies to phospho-S6K(T389), S6K, phospho-S6(S240/244), S6, phospho-AKT(S473),

phospho-AKT(T308), AKT, phospho-ERK1/2, ERK1/2 and mTOR were products of Cell Signaling Technology (Cat. #9206, #9202, #2215, #2217, #4060, #4056, #9272, #9101, #5013, #2972). Two types of antibodies were used for detecting myc (9E10), Santacruz (Cat. sc-40) and Bethyl (Cat. A304-315A). Antibody to actin was obtained from Sigma Aldrich (Cat. A2228).

Establishment of LRS shRNA, myc-LRS expression cell line

Lenti-X 293T cells were seeded evenly in 100 mm dish with 10 ml of DMEM. After incubating about 12 hours at 37℃, 5% CO_2 in incubator, the cells should be prepared at 90% confluency for transfection.

Xfect Reaction Buffer, Lti-X HTX Packaging Mix 2 and Lenti-X vector DNA (1 μg/μl) were added into the first tube. Then Xfect Reaction Buffer and Xfect Polymer were added into the second tube. Next, vortexed tubes were put together in one tube. After incubation for 10 minutes at room temperature, the mixture was dropped onto cells which stated above. After 4 hours of incubation at 37℃ in 5% CO_2 incubator, 10 ml fresh complete medium was replaced and incubated for additional 48 hours. The lentiviral supernatants were filtered through a 0.45 μm filter, dropped the virus to target cells.

The cells were gradually selected by treating puromycin in few days. This cell line expresses myc-tagged LRS or LRS shRNA when doxycycline is treated on. To check how

many days are needed to express the stable level of myc-tagged LRS or LRS shRNA, we treated 1 μg/ml doxycycline on cells for 5 days.

Leucine starvation and cell stimulation

Before cells become leucine depletion state, they were rinsed with leucine-free DMEM twice. Then the cells were incubated in leucine-free DMEM for 50 minutes, exchanged the DMEM with 0.8 mM leucine for 10 minutes.

Methionine incorporation

Whatman filter paper was soaked with 5% TCA solution and dried completely. The cells seeded on 24 well plates were washed twice with serum free RPMI and treated with mixture of serum free RPMI and 1 μCi [^{35}S] methionine (1175 Ci/mmol, Perkin Elmer). Cells were incubated for 30 minutes at 37℃ in 5% CO_2 incubator. At the end of reaction, cells were washed with warm 1ⅩPBS and suction was performed twice. NaOH solution was put on the plate to lyse the cells, and the cells were collected into eppendorf tube. Then, 10% TCA solution was added to eppendorf tube. These samples were put on the dried filter paper and dried again. The filter papers were washed with 5% TCA for 10 minutes at 4℃, 3 times repeatedly. The filter papers were maintained in 100% ethanol for 20 minutes at 4℃. Finally,

dried filter papers were measured by liquid scintillation counter.

Colony formation assay

Transformed SW620 cells (induced LRS shRNA, myc-LRS) by using lentivirus were subjected to colony forming assay. 1 μg/mL doxycycline was treated on transformed cells for 3 days before experiment. And 0.5 mL of 0.8% agarose containing RPMI was placed on 12 well plates for basal layer. 3×10^3 cells were mixed with 1 mL of 0.4% low melting agarose containing RPMI for each upper layer. The media was replaced every other day. After 4 weeks of incubation, cells were fixed using mixture of 10% acetic acid and 10% methanol in double-distilled water for 10 minutes on the shaker. The fixed cells were washed with double-distilled water for 10 minutes on the shaker. The cells were stained with 0.01% crystal violet for 1 hour on the shaker. Then, the cells were destained with double-distilled water on the shaker until the background is faded out. At the end, the colonies on the plates were counted.

Xenograft tumors

To develop xenograft tumors, 1×10^7 for SW620 LRS shRNA cells and 1.3×10^7 for SW620 myc-LRS cells in 200 μl serum-free RPMI media were injected subcutaneously into

each nude mouse. Total 10 mice were involved in xenograft experiment for each group. Doxycycline (20 mg/ml) was applied in the 5% sucrose water after 2 days later from the point of cell injection. The tumor volume was measured every three days with caliper and calculated according to the formula tumor volume (mm^3) = (shorter $diameter^2$ X longer diameter) / 2. Approximately 4 weeks later, mice were sacrificed and the tumors were removed.

RESULTS

LRS is highly expressed in colorectal cancer

To investigate whether level of LRS is elevated in colorectal cancer, we performed immunohistochemistry (IHC) staining to both colorectal cancer tissue and normal tissue of 117 patients. We confirmed that LRS is expressed stronger in primary human colorectal cancer tissues than normal tissues (Figure 1A). Through the quantitative analyses of the intensity of staining, colorectal cancer tissues have approximately 4 times higher percentage of intensity score which are above stage 2 compared to normal tissues. The ratio of the IHC score is showing that the amount of LRS expression in cancer tissue is higher than normal tissue (Figure 1B and 1C). After we confirmed LRS is more expressed in colorectal cancer tissue, we also verified the relationship between LRS and the mTOR pathway. Since the previous study revealed the mTOR pathway is the major pathway for signaling of amino acid, we checked the level of phospho-S6 (pS6) which is one of the indicators for mTOR signaling pathway. Through IHC, we found that tumor tissue which expresses LRS higher, also expresses pS6 stronger (Figure 1D). High LRS positive staining is statistically meaningful in correlation with high pS6 positive staining. Also low LRS positive staining revealed low pS6 positive staining (Figure 1E). These results demonstrate that LRS has the ability to promote

the mTOR signaling pathway.

Increase of LRS enhances mTORC1 activity and tumor growth

Above, we confirmed LRS is highly expressed in colorectal cancer tissue. To evaluate the pathological effect of LRS upregulation, we expressed myc-tagged LRS (myc-LRS) in SW620 cells. To check how many days are needed to express the stable level of myc-LRS, we treated 1 μg/ml doxycycline on cells for 4 days. We also confirmed that LRS is saturated after 2 days from treating doxycyline (Figure 2A). These cells were used for checking whether increased LRS effects on the mTORC1 signaling pathway. Highly expressed LRS made the mTOR pathway active, even without leucine stimulation (Figure 2B). We also checked the amount of protein synthesis by [^{35}S] methionine incorporation. The amount of protein synthesis was statistically larger in myc-LRS expressed SW620 cells (Figure 2C). Likewise, highly LRS expressed cells show the faster growth than those whom expressing normal level of LRS (Figure 2D). After the confirmation, we also monitored the functions of LRS as oncogenic factors through colony formation assay. The result from the colony formation assay indicates the cells became more oncogenic, showing the higher number of colonies (Figure 2E). These results suggest that LRS is deeply involved in the mTORC1 signaling pathway and have effects on formation of tumor as well.

LRS upregulation promotes tumorigenic transformation in xenograft model

To further evaluate the oncogenic potential of LRS in vivo, we injected SW620 cells into mice. The injected cells were expressing myc-LRS when doxycycline was orally taken in water by mice. The tumors were harvested 20 days later after the cell injection. All of the mice made a tumor mass in both groups. We confirmed that the cells expressing myc-LRS developed larger tumors than the cells which express the normal level of LRS. The tumor volume shows a meaningful difference between the two groups (Figure 3A and 3B). Histologically, myc-LRS expressed tumors display evaluated S6 phosphorylation and malignant phenotypes (e.g. higher cell density, bigger cell size and nuclear variability) (Figure 3C). These results show that upregulated LRS is sufficient to develop a tumor mass in vivo as well as in cell level.

Downregulation of LRS suppresses mTORC1 activity and tumor growth

We confirmed above, SW620 cells tend to have oncogenic characteristics when LRS is highly expressed. Through further examination, we expressed GFP-tagged LRS shRNA (GFP-LRS shRNA) in SW620 cells. To check how many days are needed to express the stable level of GFP-LRS shRNA, we treated 1 μg/ml doxycycline on cells for 5 days. Then we confirmed that level of LRS is strongly suppressed after 3 days from treating doxycyline (Figure 4A). These cells were used for checking whether downregulated LRS affects on the

mTORC1 signaling pathway. The cell line, LRS is strongly knocked down, shows mTOR pathway suppression, during the leucine stimulation (Figure 4B). We also checked the amount of protein synthesis by [^{35}S] methionine incorporation. The amount of protein synthesis was extremely inhibited in GFP-LRS shRNA expressed SW620 cells (Figure 4C). Likewise, GFP-LRS shRNA expressed cells shows slower cell growth than which does not express (Figure 4D). After the confirmation above, we monitored the functions of LRS as an oncogenic factor through colony forming assay. The result from colony forming assay indicates the cells became less oncogenic showing with lower number of colonies (Figure 4E). These results suggest that LRS is deeply involved in mTORC1 signaling pathway and affects formation of tumor as well.

LRS suppression inhibits tumorigenic transformation in xenograft model

We further verified the oncogenic potential of LRS by injecting mice with SW620 cells expressing GFP-LRS shRNA which induced by doxycycline. As a result, LRS knocked down cells formed dramatically small tumors in nude mice. Comparing the tumor volume between two groups, there is clearly visible differences though all the mice with formation of the tumor mass (Figure 5A and 5B). In addition, we confirmed GFP-LRS shRNA expressed tumors revealed decreased S6 phosphorylation and proliferation by IHC (Figure 5C). The results also indicate that LRS is sufficient to take a character as a tumor in vivo as well as in

cell condition.

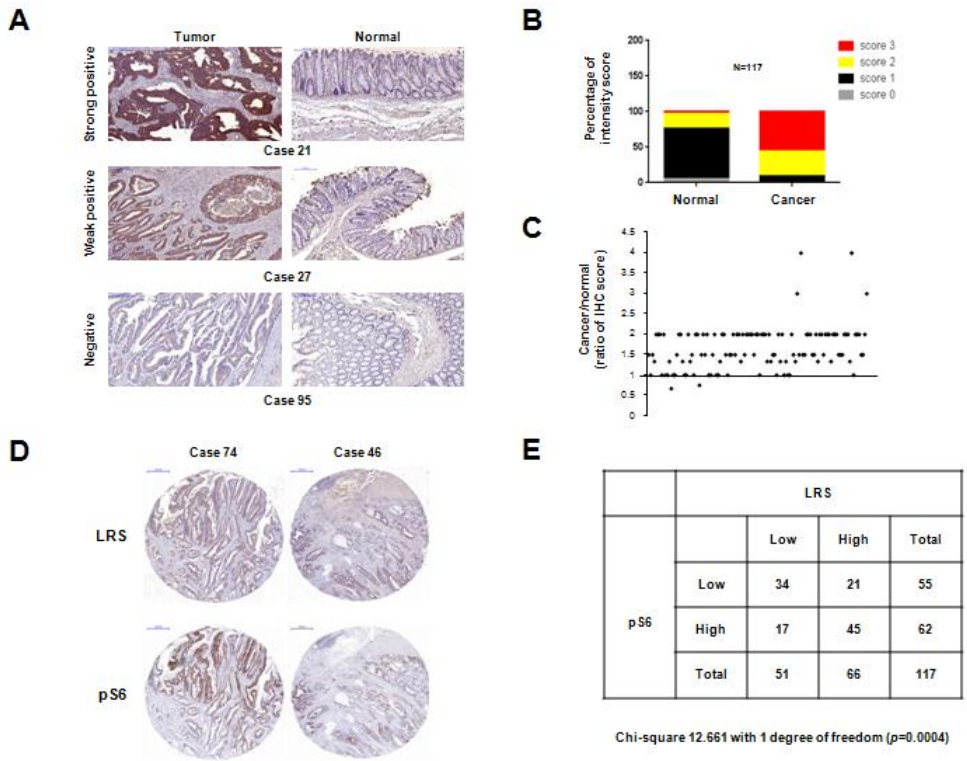

Figure 1. LRS is highly expressed in colorectal cancer tissue.

(A) Each pair of IHC staining is from the primary human colorectal cancer (CRC) tissue and normal tissue from the same patient. Shown are representatives of strong, weak and negative LRS staining of tissue.

(B) Bar graph is showing the percentage from the intensity score of LRS expression in CRC tissue and normal tissues.

(C) Each dot represents the ratio of IHC score from CRC tissue and normal tissue of each patient.

(D) Consecutive tissue sections from CRC patients were stained for LRS and phospho-S6 showing the correlation between LRS and mTOR pathway.

(E) Summary table of correlation between LRS and phospho-S6 from IHC of colorectal cancer patients.

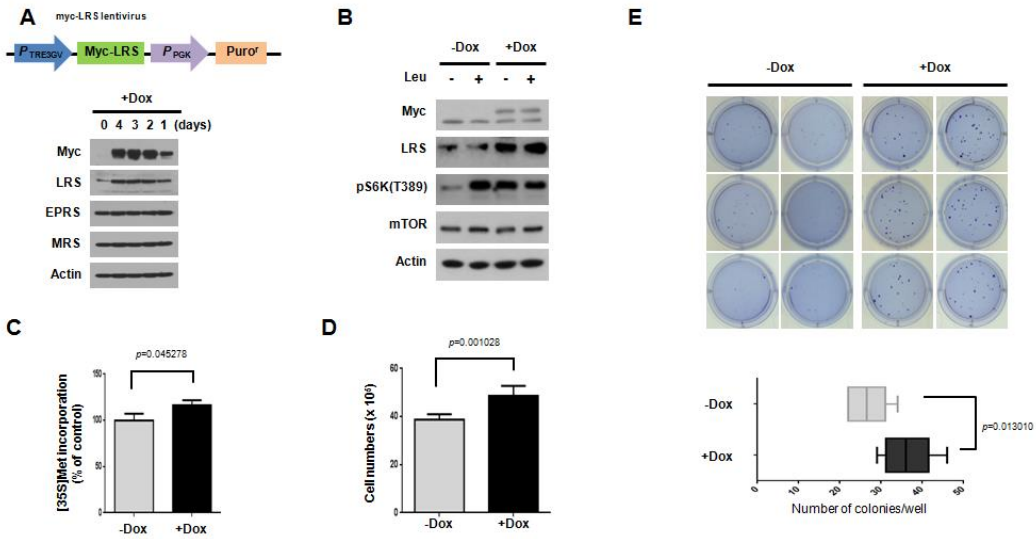

Figure 2. Increase of LRS enhances mTORC1 activity and transformation ability

(A) Myc-LRS expressed SW620 cell line was established by lentivirus system. 1 μg/ml doxycyline (Dox) was treated to induce myc-LRS for presented days.

(B) SW620 cells which induced myc-LRS by doxycycline were starved to leucine for 50 minutes and stimulated with 0.8 mM leucine for 10 minutes. The expression of phospho-S6K(T389) was analyzed by immunoblotting.

(C) SW620 cells expressing myc-LRS and empty vector were starved to serum for 30 minutes, and protein synthesis was measured with [^{35}S] methionine incorporation.

(D) SW620 cells (1×10^7) were seeded on 6 well plates for 5 days. Cell number is counted by the cell counter.

(E) Representative images are showing the increase of colony forming ability in the myc-LRS expressed cell line. Quantitative analyses of foci numbers are shown with the bar graph at the bottom.

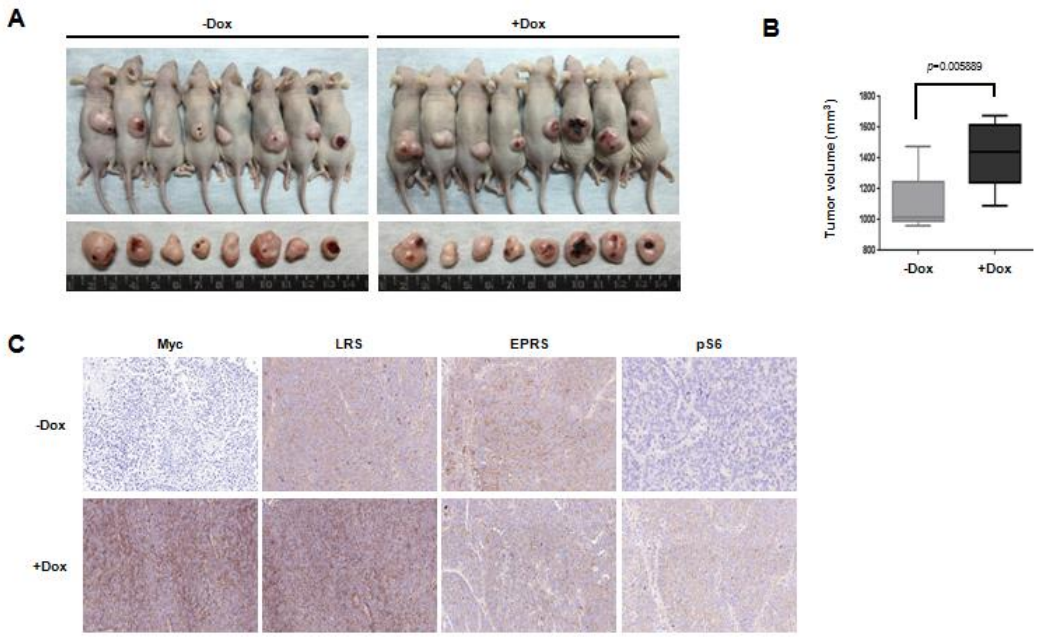

Figure 3. LRS upregulation promotes tumorigenic transformation in xenograft model

(A) Representative images of xenografts and a summary of tumor volume from mice.

(B) The volume of xenograft tumors is summarized in the bar graph.

(C) Xenograft tumors originated from LRS upregulated SW620 cells were analyzed for detecting myc, LRS, EPRS and phospho-S6 by IHC. Myc was tagged on LRS and EPRS was used as negative control.

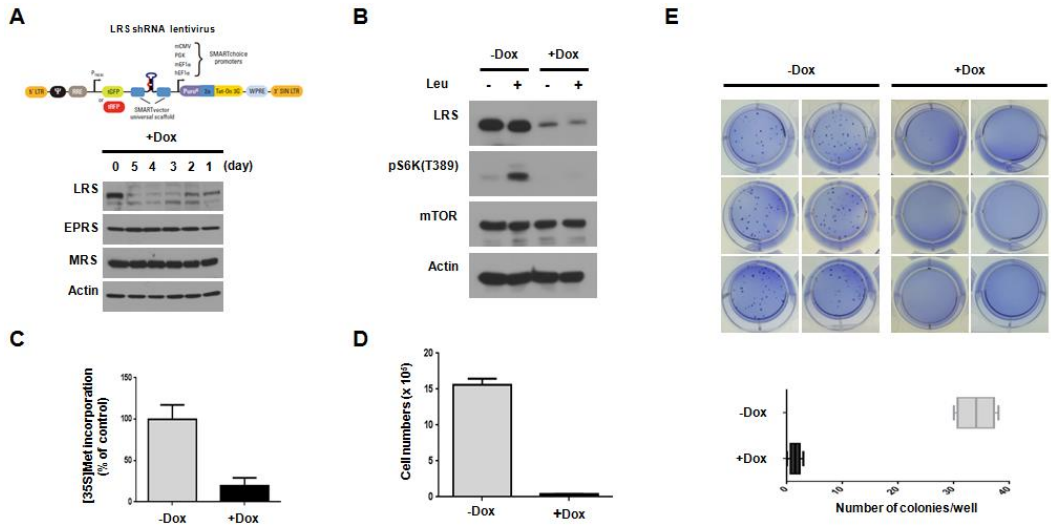

Figure 4. Downregulation of LRS suppresses mTORC1 activity and transformation abiility

(A) LRS shRNA expressed SW620 cell line was established by lentivirus system. 1 μg/ml doxycyline (Dox) was treated to induce LRS shRNA for presented days.

(B) SW620 cells which induced LRS shRNA by doxycycline were starved for leucine for 50 minutes and stimulated with 0.8 mM leucine for 10 minutes. The level of phospho-S6K(T389) was analyzed by immunoblotting.

(C) SW620 cells expressing LRS shRNA and empty vector were serum and methionine starved for 30 minutes, and protein synthesis was measured with [^{35}S] methionine incorporation.

(D) SW620 cells and SW620 cells with LRS shRNA were analyzed for cell growth. The cells (1×10^7) were seeded on 6 well plates for 5 days.

(E) Representative images are showing the decrease of colony forming ability in the LRS shRNA expressed cell line. Quantitative analyses of foci numbers are shown with the

bar graph at the bottom.

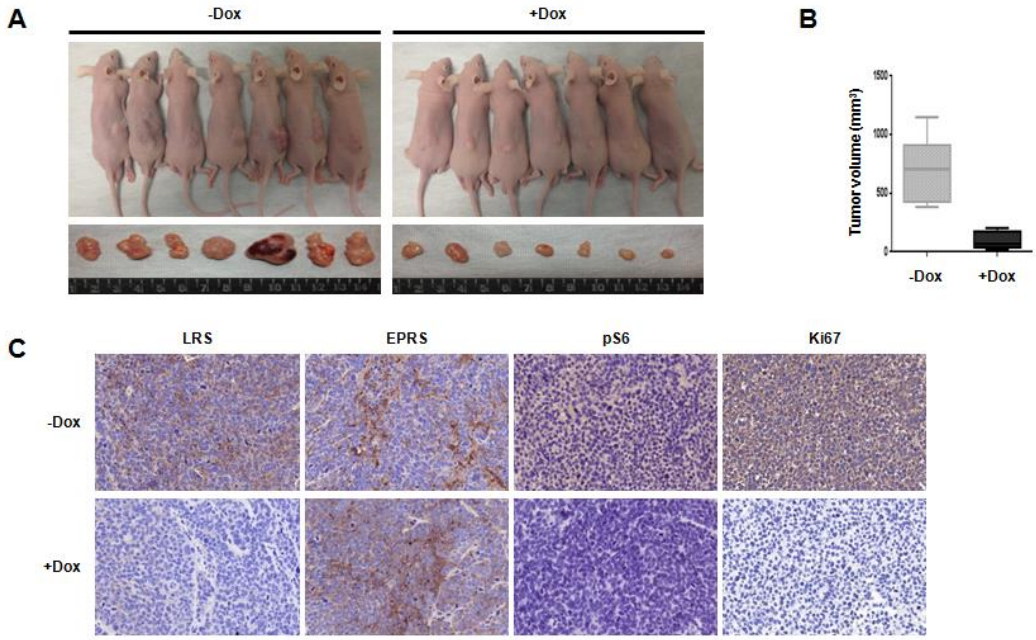

Figure 5. LRS suppression inhibits tumorigenic transformation in xenograft model

(A) Representative images of xenografts and a summary of tumor volume from mice.

(B) The volume of xenograft tumors is summarized in the right bar graph.

(C) Xenograft tumors originated from LRS knocked down SW620 cells were analyzed for detecting LRS, EPRS, phospho-S6 and Ki67 by IHC. EPRS was used as a negative control.

DISCUSSION

It has been previously reported that the leucyl-tRNA synthetase (LRS) acts as an intracellular leucine sensor which positively regulates the mTORC1 signaling pathway [2]. However, whether LRS actually has tumorigenicity has remained to be elucidated. In this study, we described LRS promotes the mTOR signaling pathway and that leads to various features of tumorigenicity.

In colorectal cancer patients' tissue, we found higher level of LRS expression compared to normal tissue and there is correlation between LRS and mTOR. The statistical analyses show the linkage between LRS and mTOR signaling pathway in colorectal cancer patients (Figure 1). In addition, we screened various colorectal cancer cell lines to confirm the expression level of LRS is higher than normal colorectal cell line. We found they also have a correlation between LRS and mTOR (Not published). These results are consistent with the previous report demonstrating leucine, one of branched amino acid, stimulates LRS and activates mTOR signaling pathway [2].

Subsequently, our functional studies show the influence of LRS on tumorigenicity. Upregulation of LRS promoted various cancer specific features including cell growth and proliferation and anchorage independent growth. Notably, colorectal cancer cells with higher level of LRS showed the activation of mTOR signaling pathway even without leucine

stimulation (Figure 2). However, downregulated LRS showed the remarkable suppression of cancer features showing the slower cell growth and proliferation (Figure 4). These results are sustained in mice xenograft model (Figure 3, 5).

Taken together, our data demonstrate LRS leads to cancer specific phenotypes through mTOR signaling pathway in colorectal cancer. Moreover, we found LRS affects to tumorigenicity in vivo as well as in cell environment. Therefore, we suggest LRS itself as a new molecule that leads to the oncogenic phenotypes through the mTOR signaling pathway in colorectal cancer.

mTOR pathway is well known signaling pathway which is activated in various cancer types [14, 16]. For this reason, there were numerous movements to treat the cancer through the regulation of mTOR pathway [13]. Two rapalogues, temsirolimus and everolimus, are already clinically applied to cure the cancer patients [17]. However, mTOR inhibitors have limitations on colorectal cancer showing the therapeutic resistance caused by mTOR mutations. Therefore, it is necessary to find an effective way to target the mTOR pathway of colorectal cancer tumorigenesis, compared to how rapalogues work. Here, our findings can be the alternative way to suppress the mTOR pathway through LRS.

It can be investigated and developed as a potent drug target, alternative to existing mTOR inhibitor, after validation. Further studies could suggest the effective drug for treating colorectal cancer by regulation of cancer homeostasis through the LRS-mTOR signaling pathway.

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요약 (국문초록)

대장암 유발에 관여하는 Leucyl-tRNA synthetase의 기능적 의의

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최근 5년 간의 통계에 따르면 대장암은 높은 발병률과 사망률을 보이는, 위험성이 높은 암이다. 그럼에도 불구하고, 현재 대장암을 치료하기 위해 임상에서 사용하고 있는 약물치료법은 효과적으로 작용하지 못하고 있다. 대표적으로 대장암 치료에 사용되는 약물인 5-FU는 암세포뿐 아니라 정상 세포까지 공격하여 적지 않은 부작용을 나타내고 있다. 따라서 우리는 암세포에 특이적으로 활성화 되어있는 신호경로를 겨냥하는 것이 효과적일 것이라는 판단하에 대장암 조직에 활성화 되어있는 신호경로를 탐색해보았다. 그 결과, 대장암에 mTOR 신호경로가 활성화 되어있다는 사실을 확인하였다. 하지만 대장암은 mTOR 자체의 변이가 높은 암 종류로서, mTOR 신호경로를 억제하기 위해 널리 사용되고 있는 약물인 rapamycin에 대한 내성이 가장 높은 암이기도 하다. 따라서 대장암에 활성화 되어있는 mTOR 신호경로를 효과적으로 억제하기 위해서는, rapamycin과는 다른 메커니즘으로 mTOR 신호경로를 억제할 수 있는 방법을 찾아야 한다.

한편, 앞선 연구에서 leucyl tRNA synthetase (LRS)는 mTORC1 신호경로에 대해 신호를 전달할 수 있도록 아미노산을 인지하는 전달자로서의

역할을 한다는 보고가 있었다. 따라서 이 논문을 통해, 우리는 LRS가 mTOR 신호경로를 활성화 시킴으로써 대장암의 유발과 증식에 어떤 영향을 미칠 수 있는지를 알아보고자 하였다.

먼저 대장암 환자로부터 유래된 조직을 통해, 대장암 조직에는 mTOR 뿐 아니라 LRS도 과발현되어 있다는 사실을 확인할 수 있었다. 이들을 통계적으로 처리해 보았을 때 두 분자 사이에 유의한 연관성이 있다는 사실을 알 수 있었다.

LRS가 대장암의 생성 및 증식에 미치는 영향을 알아보기 위해 대장암 세포주에 LRS를 과발현 시켜 보았다. 그 결과 LRS가 과발현 되었을 때, 상대적으로 증가한 단백질 합성과 세포 증식을 관찰할 수 있었다. 또한 LRS가 아미노산을 인지하여 mTOR 신호경로에 전달하는 매개체로 작용한다는 연구 결과가 보고 되어 있었으므로, 이에 착안하여 과발현된 LRS가 mTOR 신호경로에 어떤 영향을 미치는지 확인해보았다. 흥미롭게도, LRS가 과발현 되어있는 대장암 세포주에서는 류신 결핍 상태임에도 불구하고 LRS의 과발현만으로 mTOR 신호경로가 활성화 되는 것을 확인할 수 있었다.

LRS 발현양을 줄인 암세포를 통해, LRS의 부재로 인한 세포 증식의 감소와 단백질 합성량의 감소를 뚜렷하게 확인할 수 있었다. 더불어 LRS의 발현을 감소시킨 세포에서는 류신이 존재함에도 불구하고 mTOR 신호경로를 활성화시키지 못하는 것을 확인할 수 있었다.

쥐 모델 실험을 통해서도 세포 수준에서 얻었던 결과와 같은 결과를 얻을 수 있었는데, 이는 생체 내에서도 LRS가 mTOR 신호 경로의 활성화에 영향을 미치며 나아가 LRS를 조절하여 암을 억제할 수 있을 것이라는 사실을 다시 한번 시사한다.

따라서 대장암에 과발현 되어있는 LRS는, 암 특이적으로 발현되어 있는 mTOR 신호경로와 밀접한 상관관계를 가지고 있으며 mTOR 신호경로를 효과적으로 조절할 수 있는 또 하나의 강력한 방법이라고 할 수 있다. 다시 말해, mTOR 억제제인 rapamycin에 대한 내성이 있는 환자들에게 있어 LRS는 mTOR를 효과적으로 제어할 수 있는 새로운 방안이 될 수 있다.

이 같은 결과를 통해, LRS가 암세포의 성장과 증식에 영향을 미치는 조절분자로서의 측면을 확인해 볼 수 있었다. 이는 LRS가 새로운 약물표적으로서 충분한 가능성을 가지고 있다는 것을 제시해 준다.

주요어 : 류신-tRNA 합성효소 (LRS), mTOR, 대장암, tumorigenicity, therapeutic target

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