약학석사학위논문

핵 수용체 RORα의 IL-6R 신호전달 조절을 통한 간세포 증식 억제 효과

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2017년 2월

서울대학교 대학원
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2017년 2월

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ABSTRACT

Suppression of hepatocyte proliferation through RORα-mediated IL-6R signaling pathway

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Nonalcoholic fatty liver disease (NAFLD) is classified into simple steatosis and steatohepatitis, which can progress to hepatocellular carcinoma. Fat accumulation within hepatocytes results in ROS production and triggers liver inflammation In an inflammatory region of liver, IL-6, which is one of the major inflammtory cytokines, binds to membrane-bound IL-6R on hepatocyte and IL-6R stimulation leads to the activation of JAK/STAT3, and induces upregulation of genes involved in compensatory proliferation. Therefore, inhibition of IL-6R signaling is important for regulation of liver malignancy. Retinoic acid receptor related orphan receptor α (RORα) is related various liver metabolism diseases including NAFLD. Recently, It is reported that RORα could be a potential tumor suppressor gene in liver cancer, but the specific mechanism is elusive. To identify the IL-6 signaling component regulated by RORα, I used RORα overexpression

adenovirus in primary mouse hepatocytes. The data showed that RORα overexpression decreased expression of IL-6Rα. Moreover, I observed that RORα overexpression decreased the activation of JAK2 and STAT3, the downstream factors of IL-6Rα. Also, Chromatin Immunoprecipitation followed by sequencing (ChIP-seq) in public database showed five RORα binding signals on the intron region of *IL-6Rα* gene, and it was identified that RORα bound to one of the five region followed by histone deacetylation by ChIP assay. Next, I tested effects of RORα after partial hepatectomy model. I found that upregulation of IL-6R signaling resulted in facilitating hepatocyte proliferation in liver-specific RORα knockout mice. Also, activation of IL-6R signaling increased diethylnitrosamine (DEN)-induced tumorigenesis in liver-specific RORα knockout mice. These findings revealed that RORα is a novel therapeutic target for compensatory proliferation-induced liver cancer.

Key Words : RORα, IL-6Rα, partial hepatectomy, Diethylnitrosamine

Student Number : 2015-21869.

CONTENTS

LIST OF FIGURES

LIST OF TABLE

LIST OF ABBREVIATIONS

DEN	Diethylnitrosamine
HCC	Hepatocellular carcinoma
IL-6	Interleukin-6
JAK	Janus kinase
LKO	liver-specific knockout
PHx	Partial hepatectomy
RORα	Retinoic acid receptor related orphan receptor α
STAT3	Signal transducer and activator of transcription 3

Ⅰ. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a spectrum of liver disease ranging from liver steatosis and steatohepatitis, and hepatocellular carcinoma (Starley et al., 2010). Accumulation of lipid within hepatocyte induces reactive oxygene species, resulting in hepatocyte apoptosis which leads to the recruitment and activation of inflammatory cells (Sakaguchi et al., 2011). Substances released by dying hepatocytes and proinflammatory cytokines produced by immune cells give rise to inflammation-driven compensatory proliferation, which plays an important role in hepatocellular carcinoma (HCC) development (Baffy et al., 2013). HCC is the fifth most common cancer and the second most common cause of cancer-related deaths. In less developed countries, more than 80% of HCCs are typically caused by chronic hepatitis B and C. However, in developed countries including the United States, Japan, Canada, and Europe, the incidences of chronic hepatitis B and C are decreasing whereas that of NAFLD is increasing (Michelotti et al., 2013). Despite NAFLD increases the risk of liver cancer, the mechanism of NAFLD-induced HCC is still elusive.

IL-6, a multifunctional cytokine produced predominantly by activated macrophages, is not only involved in immune responses but in cell survival, apoptosis, and proliferation . IL-6 binds to the IL-6 receptor α (IL-6Rα) subunit and subsequently induces dimerization of gp130, which is associated with IL-6Rα, and initiates intracellular signaling (Hunter et al., 2015). IL-6 signals trigger activation of JAK/STAT, MAPK, and PI3K/AKT. In particular, STAT3, the major

mediator of IL-6 signaling, is closely related to many cancers through transcriptional regulation of cell proliferation and survival-related-gene (Mauer et al., 2015). IL-6 levels are elevated in steatohepatitis patients (Dogru et al., 2008), and also HCC patients show a high serum level of IL-6 (Metwaly et al., 2012). Obesity, one of the major risk factor for NAFLD, is also associated with high risk of liver cancer (calle et al.,2004), and it is demonstrated that IL-6 is required for the development of obesity-induced HCC (Park et al., 2010). Moreover, Naugler et al. showed DEN-induced tumorigenesis was markedly reduced in IL-6 null mice. These observations suggest that IL-6 could be a key linker between early event NAFLD and liver tumorigenesis. Thus, IL-6 signaling pathway seems to be an important target for the HCC treatment.

Retinoic acid receptor related orphan receptor α (RORα) is one of the nuclear hormone receptor superfamily that mediates various physiological actions in many tissues. RORα is activated by ligand binding followed by nucleus translocation (Jetten *et al.* 2009). Notably, it is well-known that RORα plays a pivotal role in liver diseases. RORα attenuates many metabolic diseases, including NAFLD and artherosclerosis (Kim *et al.*, 2012). Also, it was demonstrated that RORα regulates expressions of inflammatory cytokines, including TNF α and IL-1β in kupffer cells, the liver residence immune cells. (Han *et al.*, 2014). According to overall and disease-free survival in HCC patients, patients with low RORα expression had shorter overall and disease-free survival rates than patients with high expression (Fu et al., 2014). This results indicate that RORα could have the potential to suppress HCC development. But the specific mechanism of RORα -mediated HCC suppression remains unknown. In this study, I

demonstrated the tumor suppressive effect of RORα through regulation of IL-6R signaling.

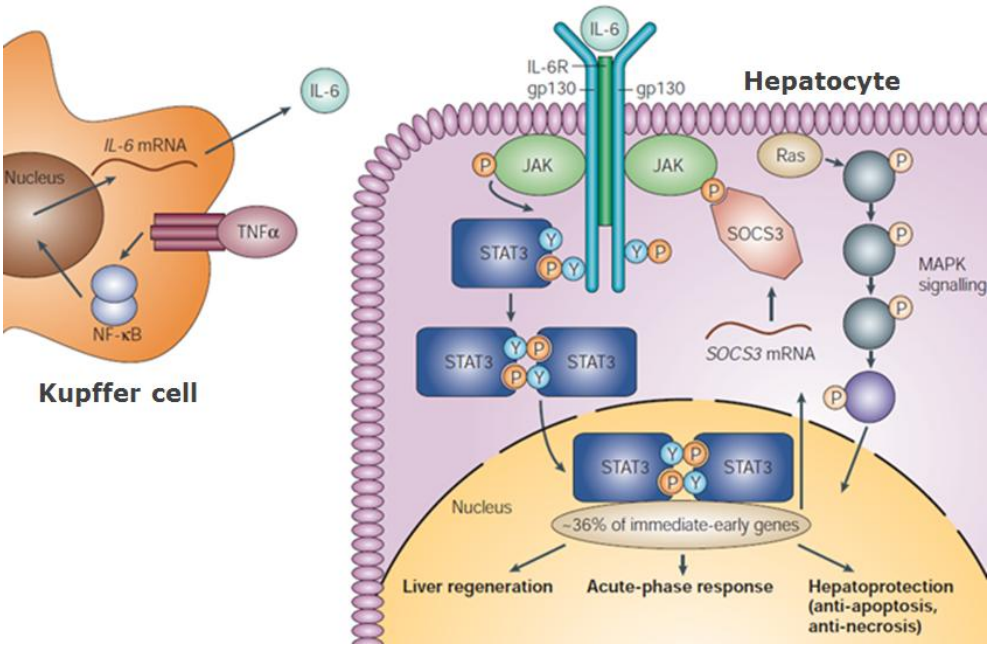

Figure 1. IL-6/STAT3 signaling pathway in liver

The schematic illustration of IL-6/STAT3 signalling. IL-6 is produced by kupffer cells through the nuclear factor (NF)-kB pathway. IL-6 binds to the IL-6 receptor on hepatocyte, which interacts with two subunits of gp130, and activates Janus kinase (JAK). Activated JAK triggers downstream signaling pathway including the mitogen-activated protein kinase (MAPK) pathway and the signal transducer and activator of transcription (STAT)3 pathway. The STAT3 transcription factor dimerizes and translocates to the nucleus. In the liver, nuclear STAT3 activates transcription of proliferation-related genes and anti-apoptotic genes (Adopted from Taub et al., 2004).

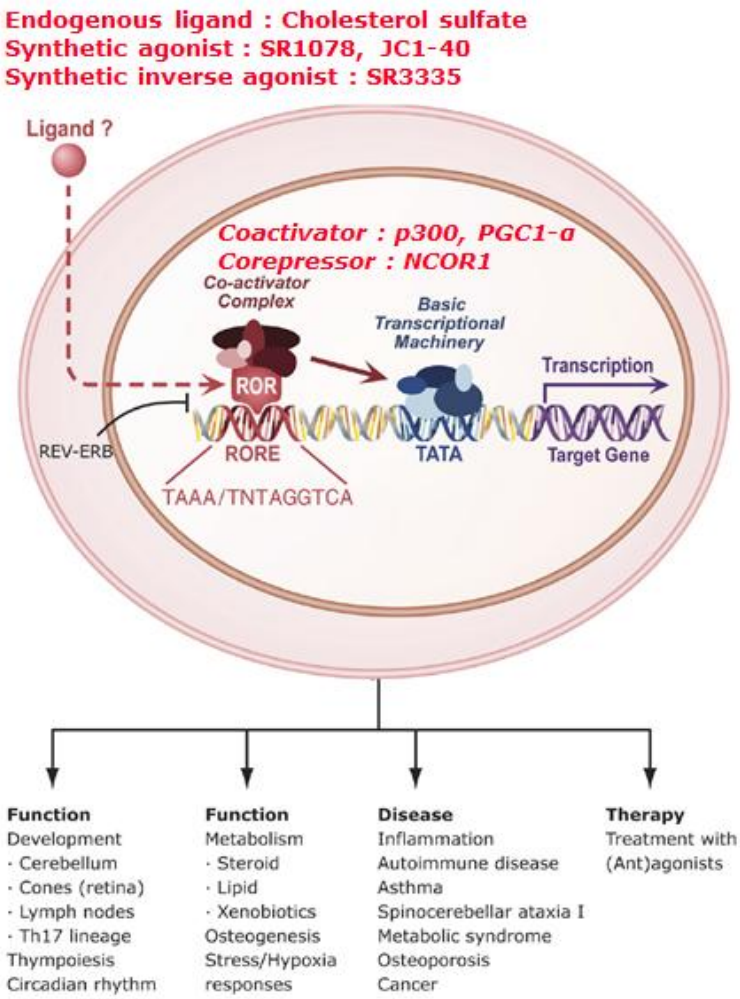

Figure 2. Physiological functions and roles in diseases of RORα

RORα is activated by ligand binding and binds as monomer to ROREs consisting of consensus core motif. RORα interacts with co-activators or co-repressors and regulates target genes expression. RORα is related to various physiological processes and many diseases including metabolic syndrome, cancer, autoimmune disease, and inflammatory responses (Adopted from Jetten *et al.*, 2009).

Ⅱ. PURPOSE OF THE STUDY

The roles of IL-6 signaling are well-known in various inflammation-driven diseases including cancers. Various drugs that block the IL-6 signaling are worldwide used in clinical practice. Drugs that target IL-6, IL-6R, or downstream signaling blocker show strong efficacy in many diseases. In the case of liver cancer, however, only IL-6 downstream signaling blocker shows efficacy in HCC patients. Because the signaling blocker is a multitargeting kinase inhibitor resulting in suppression of other signaling pathways, it is nessesary to find new target for inhibiting IL-6 signaling pathway. Nuclear receptor RORα is a potential tumor suppressor of HCC, but the mechanism is not fully understood. In the previous study, we found out that RORα suppressed phosphorylation of STAT3, one of the mediators of IL-6 signaling. In this study, I aimed to identify the IL-6 signaling component which is regulated by RORα. To investigate how RORα regulates IL-6 signaling pathway, I used RORα overexpression and ChIP assay approach in primary mouse hepatocytes. Moreover, to test the roles of RORα in HCC development, I evaluated hepatocyte proliferation after partial hepatectomy and assessed HCC prgression after diethylnitrosamine injection in liver-specific RORα knockout mice. The present study aims to demonstrate the tumor suppressive effect of RORα through regulation of the IL-6R signaling.

Ⅲ. MATERIALS AND METHODS

1. Liver-specific RORα knockout mice

Liver-specific RORα knockout mice were generated in previous study. Briefly, the RORα flox/flox embryo, in which the exon 4 of the *ROR α* allele was flanked with loxP sites, was obtained from the Institut Clinique de la Souris (Illkirch, France) and the mutant mouse was generated by in vitro fertilization (Korea Research Institute of Bioscience and Biotechnology). RORα flox/flox mice were intercrossed with Alb-Cre mice to produce liver-specific RORα knockout mice. They were housed in an air-conditioned room at a temperature of 22-24℃ and a humidity of 37-64%, with a 12 h light/dark cycle.

2. Cell culture and cell treatment

Primary mouse hepatocytes were isolated from eight-to-ten-week-old, male C57BL/6N mice (Orient bio, Korea). The liver perfusions were done by collagenase type IV (Sigma aldrich, St Louis, MO). The cells were plated at collagen-coated plates and maintained under 5% CO_2 at 37℃ in Medium 199/EBSS (M199/EBSS; SH30253.01; Hyclone; USA) supplemented with 10% fetal bovine serum (FBS, Welgene), dexamethasone, HEPES. Mouse IL-6 (406-ML-005) was obtained from R&D system. Human RORα1 recombinant adenovirus constructions were described previously (Kim et al., 2012).

3. Partial hepatectomy

The surgery was kidnly provided by prof. Seong JK (Seoul National University, Korea). Briefly, Animals were anesthetized using

isoflurane followed by ligation and resection of median and left liver lobe. For sham operation, mice were anesthetized and subject to laparotomy, and then the wound was closed. 3 h or 24 h later, mice were sacrificed and the remnant lobes were harvested.

4. Diethylnitrosamine-induced hepatocellular carcinoma

For inducing hepatocellular carcinoma, two-week-old male liver-specific RORα knockout mice and flox/flox littermates were administrated 25 mg/kg of diethylnitrosamine (DEN; N0756; Sigma aldrich, MO, St Louis) by inraperitoneal injection and sacrificed at 10 months after injection. Animal experiments were conducted in accordance with guidelines of Seoul National University Animal Care and Use Committee.

5. Chromatin immunoprecipitation assay

ChIP assays were performed as described previously (Kim et al., 2012). Briefly, nuclear lysates were sonicated, and the lysates were immunoprecipitated using specific antibodies and normal IgG antibodies for overnight at 4°C. DNA was extracted by phenol-chloroform extraction and amplified by PCR using specific primer as described in Table 1. PCR products were resolved in 2.5% agarose gel.

6. Western blot assay

Cells were washed with cold 1xPBS and harvested with a RIPA lysis buffer (25 mM Tris-HCl, 150 mM NaCl, 0.1% sodium dodecyl sulfate, 1% Triton X-100, 1% deoxycholate, 5 mM EDTA) supplement with a protease inhibitor cocktail (11.836.153.001, Roche, Switzerland) and a

phosphatase inhibitor (4906845001, Roche, Switzerland), using a cell scraper. After 30 minutes of incubation on ice, lysates were centrifuged at 14,000 rpm for 10 minutes, 4℃. Supertenant was seperated and quantified through BCA Protein Assay Kit (23225, Pierce, USA). Protein samples were loaded in a 6%-10% gel. After SDS-polyacrylamide gel electrophoresis, proteins were transferred onto a 0.45 μm polyvinylidene difluoride (PVDF) membrane by semi-dry transfer method. 5 $_{w/w}$% non-fat dry milk in PBS with 0.1% Tween-20 (PBS-T) was used for blocking membrane for 1 hours under room temperature. After blocking, membranes were incubated in primary antibodies in 5 $_{w/w}$% non-fat dry milk in PBS-T overnight in 4℃. Membranes were washed 3-times with PBS-T and incubated secondary antibodies for 1 h under room temperature. Amersham ECL solution (RPN2106, GE healthcare, USA) was used for detection after washing 3-times with PBS-T for remove antibodies.

7. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA isolation was done by EASY-BLUE^{TM} Total RNA Extraction Kit (Intron Biotechnology, Korea) according to the manufacturer's protocol. Extracted total RNA was reverse-transcripted to synthesize cDNA using M-MLV reverse transcriptase (28025-013, Invitrogen). qRT-PCR was performed using SYBR Green PCR master mix (4367659, Applied Biosystems). The resulting ΔC_t values were normalized with 18s rRNA.

8. Statistical analyses

All data were statistically analyzed by using GraphPad Prism 5 (GraphPad Software, USA). Statistical analyses were performed using

non-parameteric Mann-Whitney U test or paired t-test for comparisons of data. $P < 0.05$ denotes statistical significance.

Table 1. Primer sequences used for quantitative RT-PCR and ChIP analysis

Gene		RT-PCR Primer sequences	Product length (bp)
mIL-6Rα	Forward	5′-ATC CTC TGG AAC CCC ACA C-3′	68
	Reverse	5′-GAA CTT TCG TAC TGA TCC TCG TG-3′	
mJAK1	Forward	5′-GTC CCT GAA GCC TGA GAG TG-3′	136
	Reverse	5′-CTT GAT ACC ATT GCC TCC GT-3′	
mJAK2	Forward	5′-GAT GGC GGT GTT AGA CAT GA-3′	177
	Reverse	5′-TGC TGA ATG AAT CTG CGA AA-3′	
mSrc	Forward	5′-TCC ACA CCT CTC CGA AGC AA-3′	151
	Reverse	5′-CAT GCT GAT GGC CTG TGT CA-3′	
mAbl	Forward	5′-TCG TTA CCT CCA AAG GCT GCT C-3′	82
	Reverse	5′-ATG GCG GTG TCT GGC TAT TCA-3′	
18s rRNA	Forward	5′-GTA ACC CGT TGA ACC CCA TT-3′	
	Reverse	5′-CCA TCC AAT CGG TAG TAG GG-3′	
		ChIP Primer sequences	
RORα ChIP on *IL-6Ra*	Forward	5‘-CTT AGA GGT CTT TGC CTC CTG-3’	114
	Reverse	5‘-CAC TCT CTG CTC TTC TGA TAC AT-3’	

Ⅳ. RESULTS

1. RORα suppresses IL-6 signaling pathway through regulation of IL-6Rα in primary hepatocyte

In previous study, we found that RORα inhibited phosphorylation of STAT3. In order to identify a component of IL-6 signaling pathway regulated by RORα, the factors regulating phosphorylation of STAT3 were screened. Overexpression of RORα reduced the mRNA expression level of IL-6Rα in primary hepatocyte, but did not affect the mRNA expression level of JAK1, JAK2, Src, Abl. Then, the overexpression of RORα decreased the protein expression level of IL-6Rα. Phosphorylation of JAK2 and STAT3, the downstream genes of IL-R6, were also decreased. These results indicate that RORα inhibits IL-6 signaling pathway through transcriptional regulation of IL-6Rα in primary hepatocyte.

2. Binding of RORα to IL-6Rα intron represses transcriptional activity

To investigate how RORα regulates transcription of IL-6Rα, public RORα ChIP-seq data sets were analyzed. Five ChIP-seq peaks were detected in the intron region of mouse *IL-6Rα*. ChIP analysis performed on the five ChIP-seq signals confirmed that RORα was bound to a specific intron region. The ChIP analysis also revealed that the level of acetylation at histone H3, a marker of transcriptional activation, was decreased. These data indicate that RORα is bound to the intron region of *IL-6Rα* and downregulates transcriptional activity.

3. Hepatocyte proliferation is facilitated in liver-specific RORα KO mice

70 % partial hepatectomy (PHx) is one of the most studied model of liver regeneration. During liver regeneration, hepatocyte proliferation is strongly induced through IL-6 signaling pathway. To investigate the effect of RORα on hepatocyte proliferation, I employed the murine model of 70 % PHx in liver-specific RORα knockout (LKO) mice, in which exon 4 of the *RORα* allele was deleted. At 3 h after PHx, PHx-induced IL-6Rα expression, JAK2 and STAT3 phosphorylation were observed, indicating that IL-6R signaling was activated after PHx. Notably, under basal conditions, IL-6Rα expression and phosphorylation of JAK2 were higher in LKO liver when compared to flox/flox liver. However, because of elevated basal IL-6R signaling activity in LKO liver, no further increase of IL-6Rα expression, JAK2 and STAT3 phosphorylation were detected after PHx.

Next, to examine the impact of RORα deficiency upon PHx-induced proliferation, PCNA immunostaining was performed. Expression of PCNA, one of the major proliferation marker, was more increased in LKO mice, indicating that hepatocyte proliferation is facilitated in LKO liver. Taken together, RORα deficiency could increase hepatocyte proliferation through upregulation of IL-6R signaling activity.

4. Diethylnitrosamine-induced tumorigenesis is increased in liver-specific RORα KO mice

To further verify the role of RORα in hepatocellular carcinoma (HCC) development, I assessed Diethylnitrosamine (DEN) -induced HCC using RORα LKO mice. DEN, a chemical carcinogen, induces inflammatory response following compensatory proliferation and finally results in the development of HCC. Male mice were injected with DEN 2 weeks after birth. Macroscopically, nodules were detected on both flox/flox and LKO liver 10 months after DEN injection. However, LKO mice exhibited increased maximal tumor size relative to flox/flox control. In order to investigate the role of IL-6 signaling in HCC progression, I examined the level of IL-6Rα, pJAK2 and pSTAT3. Indeed, at 10 months after DEN administration, I observed inductions in IL-6Rα expression, JAK2 and STAT3 phosphorylation in flox/flox liver. These data indicate that DEN-induced tumorigenesis is increased in LKO liver through the IL-6R signaling.

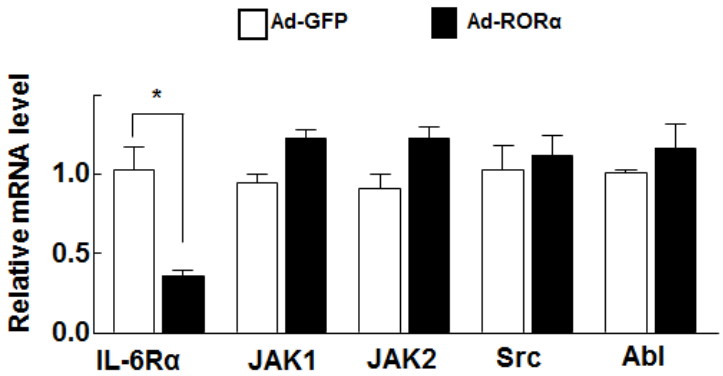

Figure 3. RORα decreases the expression of IL-6R in the primary mouse hepatocytes

Primary mouse hepatocytes were infected by 10 moi Ad-GFP and Ad-RORα1 adenovirus for 24 h. Total RNA isolated from cells was used to quantitative real-time PCR analysis of mRNA for IL-6Rα, JAK1, JAK2, Src, Abl. Data represent the means ± SEM (n=3). *$p < 0.05$.

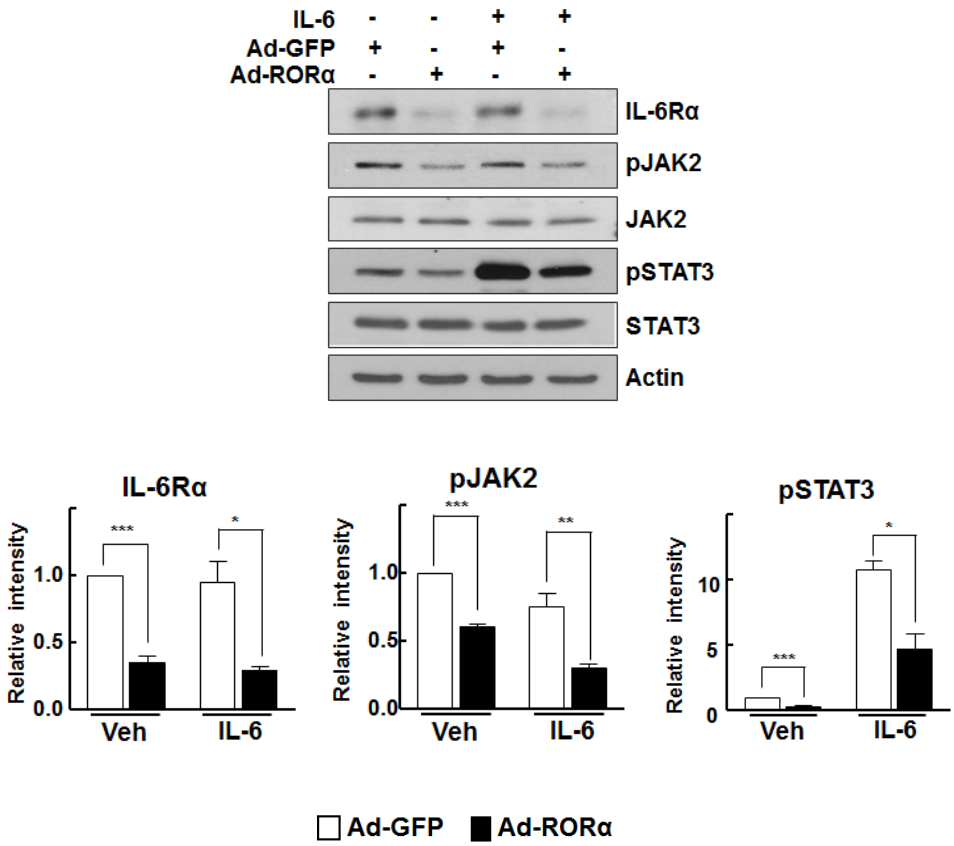

Figure 4. RORα inhibits IL-6R signaling in the mouse primary hepatocytes

Mouse primary hepatocytes were infected by 10 moi Ad-GFP and Ad-RORα1 adenovirus for 24 h and treated with vehicle, mouse IL-6 0.5 ng/ml for 4 h. The expression level of protein was measured by western blotting analysis (Top). The level of IL-6Rα, pJAK2, pSTAT3 to β-actin was quantified by the ImageJ software (Bottom). Data represent the means ± SEM (n=3). *p < 0.05, ***p < 0.0005

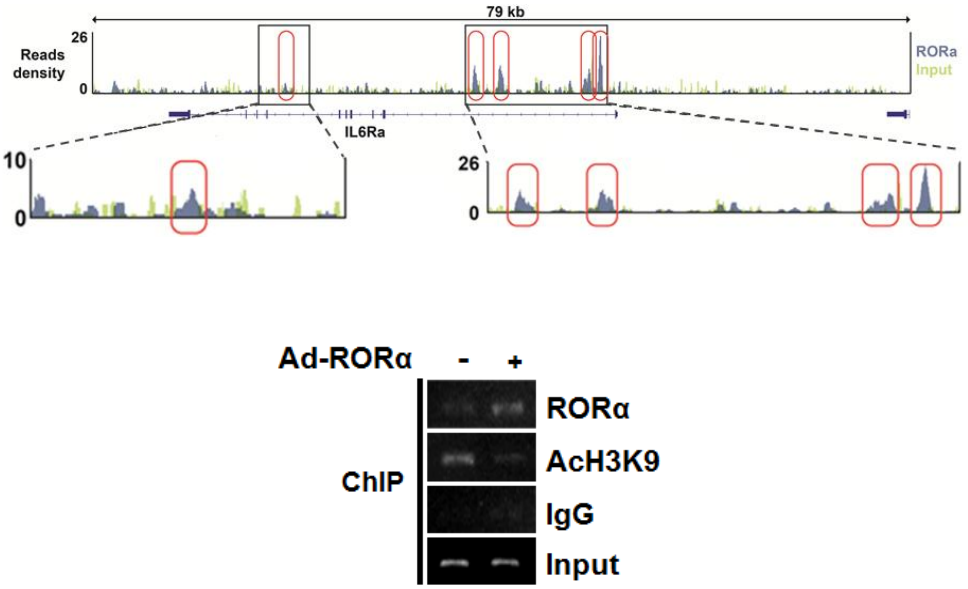

Figure 5. Binding of RORα to *IL-6Ra* intron region represses the transcription of *IL-6Ra*

Schematic representations of mouse RORα ChIP-Seq signals on *IL-6Ra* gene (Top). Mouse primary hepatocytes were infected by 10 moi Ad-GFP and Ad-RORα1 adenovirus for 24 h. DNA binding of RORα to the second proximal ChIP-Seq signal among five signals on *IL-6Ra* intron region was analyzed by ChIP assay. DNA fragments that immunoprecipitated by anti-RORα or anti-AcH3K9 antibody were amplified by PCR using primers for the second proximal signal (Bottom).

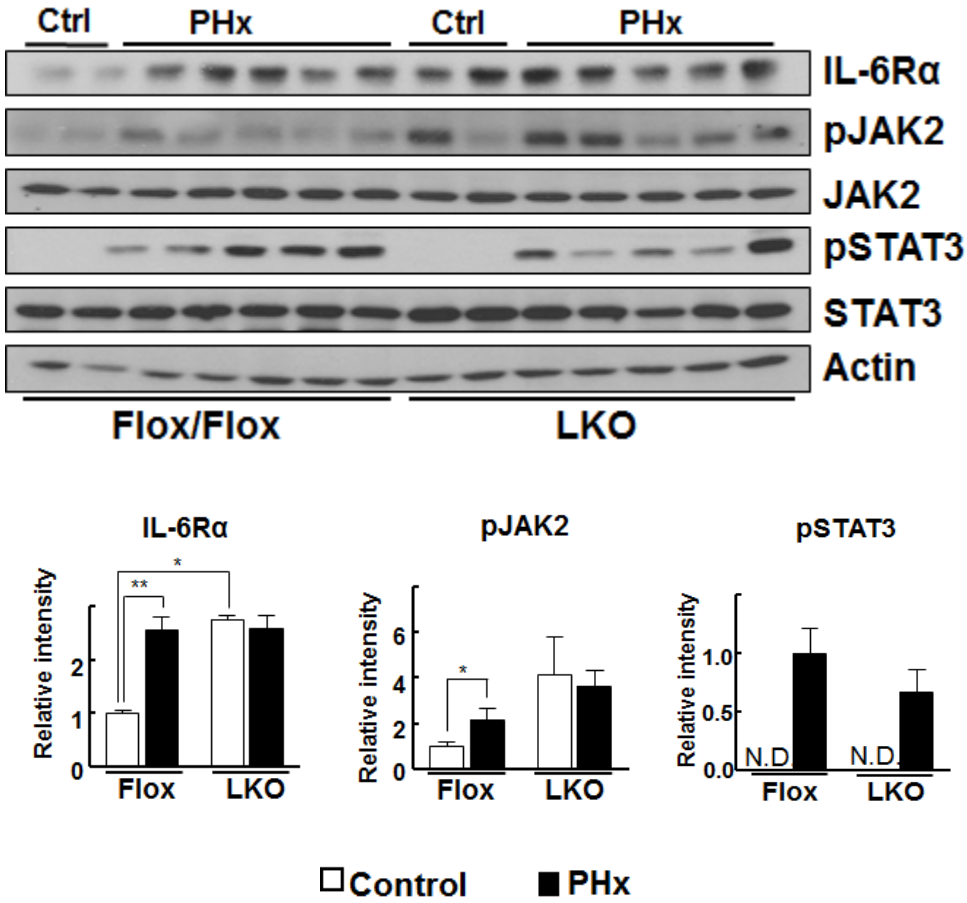

Figure 6. Expression of IL-6R signaling components at 3 h after partial hepatectomy

Liver-specific RORα knockout mice and age matched flox/flox mice littermates underwent 70 % partial hepatectomy. Animals were sacrificed 3 h after surgery (n=5 per group). The expression level of protein was measured by western blotting analysis (Top). The level of IL-6Rα, pJAK2, pSTAT3 to β-actin was quantified by the ImageJ software (Bottom). Data represent the means ± SEM (n=3). *p < 0.05, **p < 0.005, N.D. : Not detected.

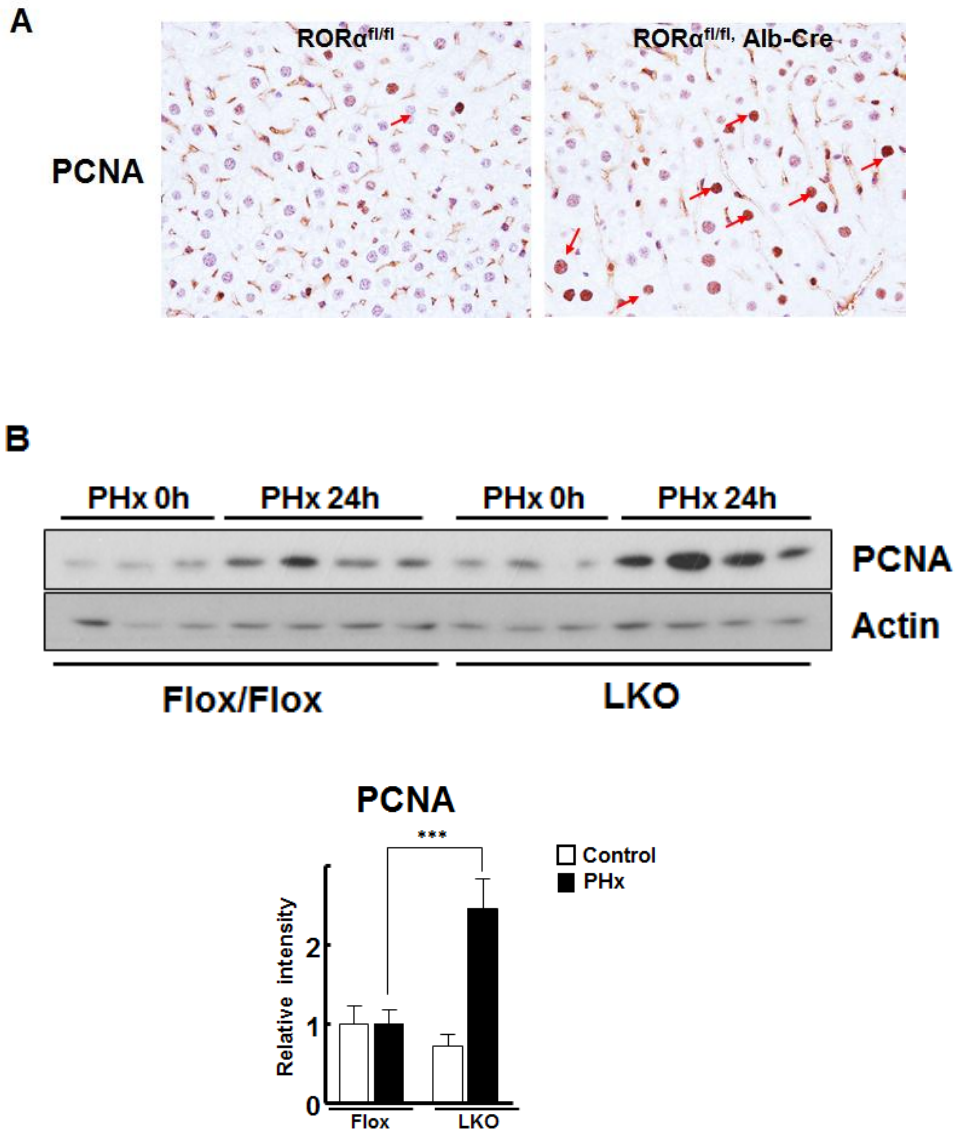

Figure 7. Hepatocyte proliferation is facillitated in liver-specific RORα knockout mice 24 h after partial hepatectomy

(A) Representative immunohistochemistry staining of liver after partial hepatectomy. (B) The expression level of protein was measured by western blotting analysis (Top). The level of PCNA to β-actin was quantified by the ImageJ software (Bottom). Data represent the means ± SEM (n=6~8). ***p < 0.0005

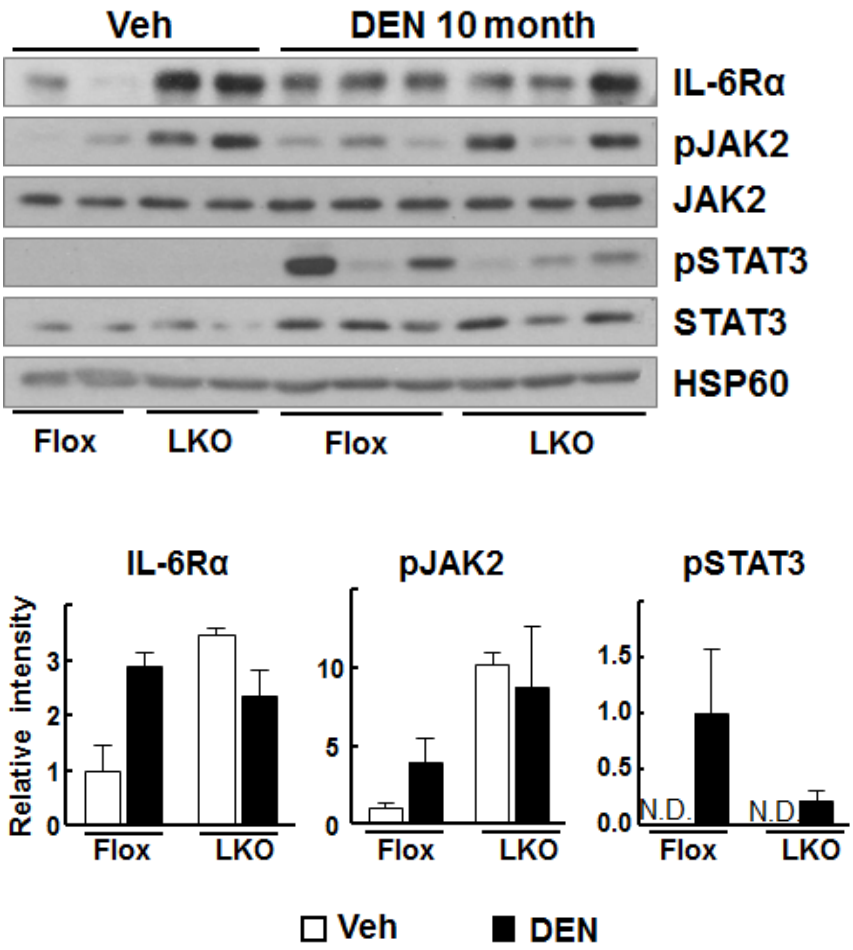

Figure 8. Expression of IL-6R signaling components at 10 months after DEN injcetion

25 mg/kg DEN was injected using intraperitoneal (I.P.) injection to liver-specific RORα knockout mice and age matched flox/flox mice littermates. Animals were sacrificed 10 months after injection (n=2~3 per group). The expression level of protein was measured by western blotting analysis (Top). The level of IL-6Rα, pJAK2, pSTAT3 to β-actin was quantified by the ImageJ software (Bottom). Data represent the means ± SEM (n=2~3).

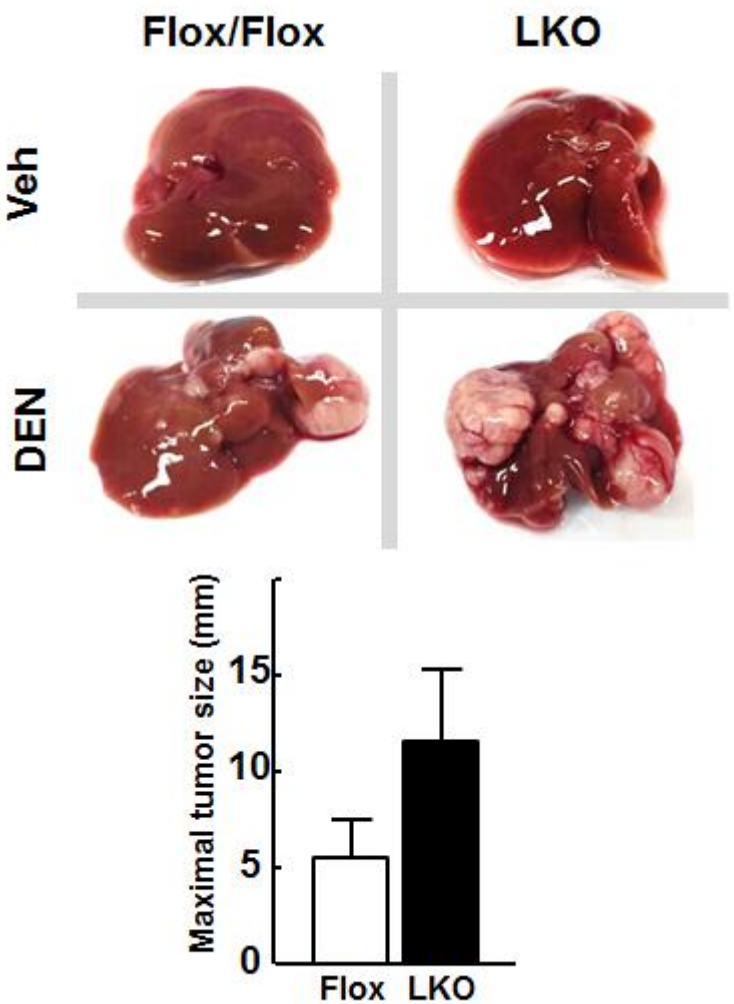

Figure 9. Liver-specific RORα knockout mice increase DEN-induced liver tumorigenesis

Representative microscopic pictures of liver from LKO mice and flox/flox littermates 10 months after DEN injection (Top). Maximal tumor size (diameter) in liver of LKO mice and flox/flox littermates 10 months after DEN injection (bottom). Data represent the means ± SEM (n=2~3).

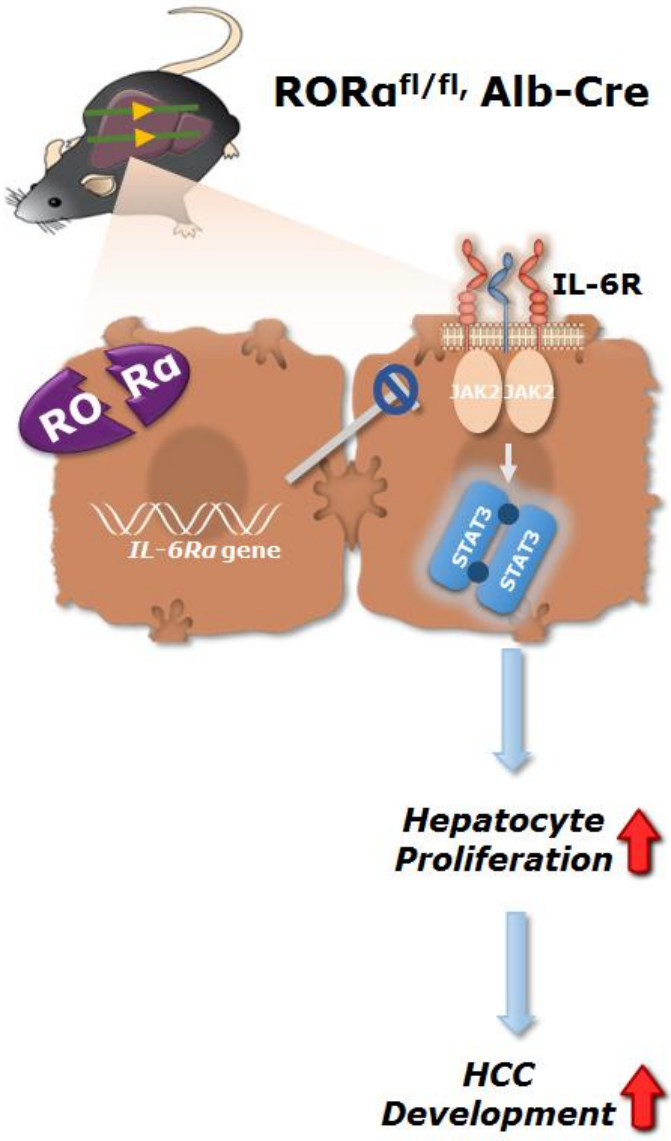

Figure 10. Schematic illustration of the effects of activated IL-6R signaling in liver-specific RORα knockout mice

In liver-specific RORα knockout mice, IL-6R signaling is activated, which results in facilitate hepatocyte proliferation after partial hepatectomy and increases DEN-induced HCC.

V. DISCUSSION

IL-6 is a multifunctional cytokine which plays an important role in various biological activities. IL-6 is involved in the cellular proliferation and survival as well as the immune response. IL-6 is an important factor in many inflammation-driven cancers including liver cancer. IL-6-deficient mice show lower incidence of HCC tumor than wild-type mice after injection of diethylnitrosamine (Naugler et al., 2007), and Giannitrapani *et., al* showed a significant increase in IL-6 level as the stage of HCC became severe. Therefore, blocking of IL-6 signaling would be a good therapeutic target for HCC. Strategies to specifically block IL-6 signaling include neutralizing antibodies to IL-6, IL-6R, JAK inhibitors, and downstream signaling inhibitors (Hunter et al., 2015). Among the IL-6 signaling blockers, only the signaling inhibitor, sorafenib, is widely used for HCC treatment (Llovet et al., 2008). But, there are several side effects in the therapeutic treatments. Thus, it is necessary to develope other strategies for the inhibition of IL-6 signaling.

RORα is a multifunctional nuclear receptor regulating circadian rhythm, development, metabolism, immune and inflammation response (Jetten et al., 2009). RORα is also involved in cellular proliferation and tumorigenesis in several tissues (Kim et al., 2011, Lee et al., 2010). In our previous studies, we revealed that RORα attenuated hepatic steatosis by regulating AMPK activity (Kim et al., 2012). Also, we identified the fact that RORα induces anti-oxidative stress enzymes and decreases steatohepatitis through reducing inflammatory response (Han et al., 2014). Recently, it was demonstrated that RORα

reduced hepatoma cell growth through regulation of glucose metabolism (Byun et al., 2015).

We previously showed that overexpression of RORα suppressed phosphorylation of STAT3, the major mediator of IL-6 signaling in mouse primary hepatocyte. In contrast, it showed that the level of STAT3 phosphorylation increased in primary hepatocyte from liver-specific RORα knockout mice. These data indicate that RORα may regulate upstream components of STAT3 in IL-6 signaling.

In this study, I demonstrated that RORα reduced transcription of IL-6Rα, and eventually could suppress hepatocyte proliferation and DEN-induced liver tumorigenesis. I clearly showed that RORα expression decreased IL-6Rα expression and inhibited IL-6R downstream signaling in primary mouse hepatocytes (Figure 4). Also, I discovered that RORα bound to the intron region of mouse *IL-6Rα* (Figure 5). Because ROR response element (RORE) is not found on the RORα binding region, I suggested the action of RORα could be mediated by other transcriptional factors. HNF4α and FOXP1, the two transcriptional factor whose putative response elements exist within the RORα binding region were selected by motif analysis. HNF4α is expressed at high levels in the liver, and regulates the large number of hepatocyte-specific genes (Walesky et al., 2015). FOXP1 is widely expressed in many tissues and especially in the liver, and it regulates glucose metabolism and cellular proliferation (Zou et al., 2015, Wang et al., 2016). The further RORα -mediated mechanism that suppresses the expression of IL-6Rα should be studied further. After partial hepatectomy and DEN injection, IL-6R signaling was well activated in flox/flox control mice. However, it was hard to detect in liver-specific RORα knockout mice

owing to upregulation of basal IL-6R signaling. Given the importance of IL-6R signaling in HCC, RORα could be a novel therapeutic target for treatment of liver cancer.

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국문초록

비알콜성 지방간질환은 간세포에 지방이 과도하게 축적된 단순지방증, 염증을 동반한 지방간염을 거쳐 간암까지 진행될 수 있는 일련의 질환군을 의미한다. 간세포에 축적된 지방이 ROS를 생성하여 염증을 유발시키고, 염증 상황에서 증가된 염증성 사이토카인으로 인한 간세포의 증식이 과도하게 일어나는 과정을 거쳐 간암이 발생한다고 알려져 있다. 대표적인 염증성 사이토카인으로 알려진 IL-6는 간세포 막에 존재하는 IL-6 수용체에 결합하여 하위 신호전달 과정을 통해 전사인자 Signal transducer and activator of transcription 3 (STAT3)를 활성화시켜 간세포의 염증 후 보상적 증식작용에 관여한다. 그러므로 이러한 IL-6 신호전달 과정을 억제할 수 있다면 과다한 간세포 증식에 따른 암화를 막을 수 있다. Retinoic acid receptor related orphan receptor α (RORα)는 비알콜성 지방간질환을 포함한 여러 대사성 간 질환을 억제한다고 알려져 있을 뿐만 아니라 몇몇 연구에서 RORα의 간암억제의 가능성을 보여주고 있지만 그 기전은 아직 확실하게 밝혀져 있지 않다. 본 연구에서는 RORα가 IL-6 신호전달 과정을 조절하는 기전을 규명하고 RORα 간 특이적 knockout 마우스를 이용한 partial hepatectomy 모델과 diethylnitrosamine (DEN) 유도 간암 모델에서 RORα의 역할에 대하여 연구하였다. 먼저, IL-6/STAT3 신호전달 과정에 관여한다고 알려진 IL-6Rα, JAK1, JAK2, Src, abl 중 IL-6Rα의 mRNA 발현이 RORα에 의해 감소하는 것을 확인하였다. 또한, RORα에 의한 IL-6Rα 발현 감소는 JAK2, STAT3의 인산화 억제를 수반하는 것을 관찰하였다. 또한, 출판된 논문의 Chromatin Immunoprecipitation followed by sequencing (ChIP-seq) 데이터 분석을 통해 RORα가 IL-6Rα의 인트론 부분에 결합하는 신호가 있는 것을 확인하였고, 실제로 그 부위에 RORα가 결합하고 히스톤 탈 아세틸화가 일어나는 것을 확인하였다. 다음으로 partial

hepatectomy를 수행하였을 때 IL-6R signaling의 활성화를 통해 간세포의 증식이 RORα 간 특이적 knockout 마우스에서 더 증가되어 있는 것을 확인하였다. 또한, RORα 간 특이적 knockout 마우스에서 활성화되어 있는 IL-6R signaling이 DEN으로 유도된 간암 발생을 더욱 증가시킬 수 있다는 것을 확인하였다. 본 연구 결과를 통해 간에서 핵 수용체 RORα가 IL-6Rα의 발현을 억제하는 것을 발견하여 RORα가 IL-6R singaling을 억제할 수 있음을 규명하였고, 간세포 재생을 유도하였을 때 RORα 간세포 증식을 억제하는 효과를 확인함으로써 RORα가 과도한 간세포 증식에 의한 암화를 막을 수 있다는 가능성을 제시할 수 있다.

주요어 : RORα, IL-6Rα, partial hepatectomy, Diethylnitrosamine
학 번 : 2015-21869