藥學碩士學位論文

An Electrophilic Analogue of Deguelin Induces p62-Mediated Autophagy in *Ras*-Transformed Human Mammary Epithelial Cells through Inhibition of STAT3 Signaling

Ras 유전자가 도입된 인체유방상피세포에서 친전자성 Deguelin 유도체의 STAT3 활성화 억제에 의한 자식작용유도

2015年 2月

서울大學校 大學院

藥學科

金 珍 太

An Electrophilic Analogue of Deguelin Induces p62-Mediated Autophagy in *Ras*-Transformed Human Mammary Epithelial Cells through Inhibition of STAT3 Signaling

지도교수 서영준

이 논문을 약학석사 학위논문으로 제출함

2015년 2월

서울대학교 대학원

약학과

김진태

김진태의 약학석사 학위논문을 인준함

위 원 장 _____(인)

부위원장 _____(인)

위 원 _____(인)

ABSTRACT

An Electrophilic Analogue of Deguelin Induces p62-Mediated Autophagy in *Ras*-Transformed Human Mammary Epithelial Cells through Inhibition of STAT3 Signaling

Jin Tae Kim

Under the supervision of Professor Young-Joon Surh

Department of Pharmacy

The Graduate School

Seoul National University

Signal transducers and activators of transcription 3 (STAT3), a member of the Janus kinase (JAK)/STAT signaling pathway, is a transcription factor that plays important roles in many aspects of cancer aggressiveness including migration, invasion, survival, self-renewal, angiogenesis, and tumor cell immune evasion by regulating the expression of multiple downstream target genes. In the previous study, a series of analogues of deguelin, a natural rotenoid present in several plants, were synthesised to develop novel STAT3 inhibitors. Among them, an electrophilic analogue SH48 bearing an α,β-unsaturated carbonyl group was found to inhibit STAT3 phosphorylation, dimerization, nuclear translocation and transcriptional activity. In this study, I confirmed the direct binding of SH48 to the STAT3 C328,

C367 and C687 residues by mass spectrometric analysis. Then, I investigated the mode of SH48-induced MCF10A-RAS cell death with special focus on autophagy. p62 and STAT3 interact each other, which may facilitate the degradation of p62. SH48 inhibits interaction between p62 and STAT3, thereby maintaining the stability of p62 in MCF10A-RAS cells. In conclusion, the deguelin analogue SH48 inhibits STAT3 signaling by direct interaction with the C328, C367 and C687 residues of STAT3.

Keywords: Signal transducer and activator of transcription 3 (STAT3), Deguelin, SH48, Autophagy, p62/SQSTM1, Atg8/LC3-II.

Student Number: 2013-21581

TABLE OF CONTENTS

LIST OF ABBREVIATION

STAT3	Signal transducers and activators of transcription 3
JAK	Janus kinase
AKT	Protein kinase B
ERK	Extracellular signal-regulated kinases
PI3K	Phosphoinositide 3-kinase
IKK	IκB kinase
IκBα	Nuclear factor kappa-light-chain-enhancer in B cells inhibitor, alpha
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
AMPK	5' AMP-activated protein kinase
mTOR	Mammalian target of rapamycin
$p27^{Kip1}$	Cyclin-dependent kinase inhibitor 1B
Rb	Retinoblastoma protein
E2F1	Transcription factor E2F1
HIF-1α	Hypoxia-inducible factor 1-alpha
VEGF	Vascular endothelial growth factor
EGF	Epidermal growth factor
PDGF	Platelet-derived growth factor
IL-6	Interleukin 6
IFN-γ	Interferon gamma
Src	Proto-oncogene tyrosine-protein kinase Src

Tyr	Tyrosine
SOCS	Suppressor of cytokine signaling proteins
PIAS	Protein inhibitor of activated STAT
Bcl-2	B-cell lymphoma 2
Mcl-1	Myeloid leukemia cell differentiation protein
MMP	Matrix metalloproteinase
ATG	Autophagy protein
P62/SQSTM1	Sequestosome 1
ULK1	Serine/threonine-protein kinase ULK1
Vps34	Vacuolar protein sorting Class III PI 3-kinase
PKR	Protein kinase R
RIP	Ribosome inactivating protein
TRAF	TNF receptor associated factors
PKC	Protein kinase C
PE	Phosphatidyl ethanol amine
DMEM	Dulbecco's modified Eagle's medium
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide
DTT	Dithiothreitol
DMSO	Dimethyl sulfoxide
siRNA	Short interfering RNA
PMSF	Phenyl methyl sulfonyl fluoride
PBST	Phosphate-buffered Saline with Tween 20
HRP	Horseradish peroxidase

EDTA	Ethylene diamine tetra acetic acid
HSP90	Heat shock protein 90
LIR	LC3 interaction region
GABARAP	Gamma-aminobutyric acid receptor-associated protein
PARP	Poly ADP ribose polymerase

LIST OF FIGURES

Introduction

Growing attention has been focused on the application of natural flavonoids in the chemoprevention and chemotherapy of malignant diseases. Several plant rotenoid compounds, such as rotenone, deguelin and tephrosin, have previously been reported to be effective against tumorigenesis, metastasis and invasion [1-3].

Deguelin is one of natural rotenoids isolated from several plants, including *Derris trifoliata Lour* (Leguminosae), *Mundulea sericea* (Leguminosae) and *Tephrosia vogelii Hook.f* (Leguminosae) [4]. It has been known as a promising preventive and therapeutic agent for diverse cancers including head and neck, breast, colon and pancreatic cancer [5]. Deguelin directly binds to the ATP-binding pocket of heat shock protein (HSP)-90 and induces suppression of signal transduction mediated by HSP-90 client proteins. These include, PI3K-Akt, IKK-IκBα-NF-κB, AMPKs-mTOR-survivin, p27-cyclinE-pRb-E2F1 and HIF-1α-VEGF which are important for the cellular response to stress and maintenance of cellular homeostasis [6-7]. Recently, suppression of a STAT3 signal pathway by deguelin was also reported in T-cell leukemia [8]. However, undesired side effects of deguelin at high doses limit its chemotherapeutic use as a potential anticancer agent [9].

In our previous study, a synthetic deguelin analogue SH48 was found to have an ability to inhibit STAT3 signaling. SH48 has an electrophilic α,β-unsaturated

carbonyl group in its structure. The compound is anticipated to interact with various biological molecules through Michael addition with nucleophilic thiol groups.

STAT3 is a member of the Janus kinase (JAK)/STAT signaling pathway [10]. It is an important regulator of many biological processes including proliferation, survival, inflammation, and immune responses. It responds to the ligands such as growth factors (e.g., EGF, PDGF, etc.) or cytokines (e.g., IL-6, IFN-γ, etc.) or non-receptor tyrosine kinases such as Src [11-13]. Activation of STAT3 involves phosphorylation at Tyr705 and dimer formation between two monomers via reciprocal phosphoTyr(pTyr)-SH2 domain interactions. Then, the STAT3 dimer translocates to nucleus and binds to a specific DNA site, thereby regulating the transcription of target genes [14]. Under physiological conditions, STAT3 activation is rapidly and transiently controlled by a negative feedback mechanism (e.g., SOCS, PIAS, etc.) to prevent dysregulated gene transcriptions [15]. However, constitutively activated STAT3 protein has been found in various types of tumors, including leukemia and cancers of the breast, head and neck, melanoma, prostate and pancreas [16]. This aberrantly activated STAT3 is believed to contribute to malignant transformation at several levels including uncontrolled proliferation through activation of several cell-cycle regulators, (e.g., cyclin D1 and c-Myc) as well as evasion of apoptosis by inducing the expression of antiapoptotic proteins (e.g., Bcl-xL, Bcl-2, Mcl-1 and surviving). STAT3 also mediates the expression of proteins involved in other hallmarks of cancer, such as invasion and metastasis

(e.g., expression of MMP) and angiogenesis (e.g., expression of VEGF) [14]. Therefore, STAT3 is considered to be an important target for the development of cancer therapeutic agents.

Autophagy is a homeostatic, catabolic degradation process whereby cellular proteins and organelles are engulfed by autophagosomes, digested in lysosomes, and recycled to sustain cellular metabolism [16]. It is thought to have a tumor suppressive or a tumor promoting functions depending on the tumor type, the stage and genetic context of cancer [17]. There are three types of autophagy including (1) macroautophagy (often simply called autophagy) in which cytoplasmic contents and organelles are encompassed in double or multimembrane autophagosomes, and subsequently delivered to the lysosome (2) microautophagy, where cytoplasm is directly engulfed by lysosomal membrane and (3) chaperone-mediated autophagy, where proteins with a specific signal sequence are transported to the lysosomal lumen by a receptor-mediated process [18]. However, only macroautophagy has been associated with caspase-independent type 2 cell death [19].

The initiation of autophagy is controlled by the ULK1 kinase complex, which integrates stress signals from mTORC1. When mTORC1 kinase activity is inhibited, autophagosome formation can occur from the phagophore. This involves Vps34, a class III PI3K that forms a complex with Beclin 1. Beclin 1 interacts with factors (Ambra, Bif1, and Bcl-2) that modulate its binding to Vps34, whose lipid kinase activity is essential for autophagy. In addition to these 2 complexes,

autophagosome formation requires the participation of 2 ubiquitin-like protein conjugation systems (Atg12 and LC3) that are essential for the formation of the phagophore [20]. In addition, the LC3 system is required for autophagosome transport and maturation. Matured autophagosomes fuse their external membranes with those from lysosomes to degrade their cargo and recycle essential biomolecules [17].

In autophagy, STAT3 inhibits PKR kinase activity and LC3 expression in human cancer cells [21-22]. Therefore, inhibition of STAT3 may potently stimulate the autophagic flux and thereby affects the fate of cells [22]. Autophagy flux can be checked by its known markers p62/ SQSTM1, LC3-II, beclin1, atg5-atg12 and so on [23]. But, previous study, only p62/ SQSTM1 and LC3-II were affected by SH48. So we have focused on relation between STAT3 inhibition and p62 flux that induced by SH48.

p62/ SQSTM1 (sequestosome 1, and herein referred to as p62) is known as a multi-domain adaptor protein that contains different kinds of protein–protein interaction motif [24]. p62, as a receptor for ubiquitinated proteins, delivers them selectively into the autophagosomes [25-28]. It functions as a scaffold protein for several signal transductions through interaction with various signaling proteins such as RIP, TRAF6, ERK, aPKC and caspase-8 [29]. p62 binds to polyubiquitin on autophagy cargo via its ubiquitin-associated (UBA) domain and the autophagosome protein light chain 3 (LC3; also known as MAP1LC3A) via its

LC3-interacting region (LIR) domain, which directs cargo to autophagosomes for degradation [30].

Atg8/LC3-II is lipidated form of LC3B which recruits lipid molecules to expand the autophagosome membrane. LC3B proteins are specifically cleaved at their C-termini by the ATG4 proteases to expose a C-terminal glycine producing the form I of the ATG8 molecule (e,g., LC3-I) [31]. This glycine residue is then conjugated to PE. The resulting PE-conjugated form II of ATG8 proteins (e,g., LC3-II) is tightly bound to the autophagosomal membranes and serves as an autophagic marker protein [32].

In this research, I investigated the possibility of interaction between SH48 and STAT3 and the association between inhibition of STAT3 signaling and induction of autophagy by SH48. This study suggests p62/SQSTM1 (sequestosome 1) as an important mediator of SH48- induced autophagy.

MATERIALS AND METHODS

Chemicals

SH48, SH42, SH43, SSI-1204 and SSI-1205 were obtained from Prof. Young-Ger Suh`s lab (College of Pharmacy, Seoul National University). Dulbecco's modified Eagle's medium (DMEM)/Ham's nutrient mixture F-12 (1:1). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum and horse serum were obtained from Gibco BRL (GrandIsland, NY, USA). Cholera toxin, hydrocortisone, insulin, human epidermal growth factor (h-EGF) and MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide] were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Antibodies against P-STAT3, p62, JAK2, P-AKT, P-ERK and PIAS were obtained from Cell Signaling (Cell Signaling Technology, Beverly, MA, USA). Antibodies against STAT3 and agarose immunoprecipitation reagent were products of Santa Cruz Biotechnolgies Co. (SantaCruz, CA, USA). Secondary antibodies, p62 short interfering RNA (siRNA), negative control siRNA, and lipofectamin. RNAi-MAX reagent were purchased from Invitrogen Life Technologies, Inc. (Carlsbad, CA, USA). All other chemicals used were of analytical or the highest purity grade available.

Cell culture

MCF10A-RAS cells were cultured in DMEM/F-12 medium supplemented with 5% heat-inactivated horse serum, 10 μg/mL insulin, 100 ng/mL cholera toxin, 0.5 μg/mL hydrocortisone, 20 ng/mL h-EGF, 2 mmol/L L-glutamine, and 100 units/mL penicillin/streptomycin. Cell lines were grown at 37℃ in a humidified air/CO2 (19:1) atmosphere. The cells were plated at an appropriate density according to each experimental scale.

MTT reduction assay

Cells were plated at a density of 5 x 10^4 cells/100 μl in 24-well plates, and the cell viability was determined by the MTT reduction assay. After incubation, cells were treated with the MTT solution (final concentration, 1 mg/ml) for 2h. The dark blue formazan crystals formed in intact cells were siRNA knockdown of p62. The human STAT3 siRNA duplex and negative control siRNA were purchased from KDR, Inc. The sequences of each siRNA were as follows: p62 siRNA-1 (sense, 5''-UGUUCUCUGAGACCCAUGAdTdT-3''; antisense, 5''-UCAUGGGUCUCAGAGAACAdTdT-3''). Dissolved with dimethyl sulfoxide (DMSO) and the absorbance at 570 nm was read using a microplate reader. Results were expressed as the percentage of MTT reduction obtained in the treated cells, assuming that the absorbance of control cells was 100%.

Western blot analysis

MCF10A-RAS cells were lysed in lysis buffer [250 mmol/L sucrose, 50 mmol/L Tris-HCl (pH 8.0), 25 mmol/L KCl, 5 mmol/L $MgCl_2$, 1 mmol/L EDTA, 2 mmol/L NaF, 2 mmol/L sodium orthovanadate, and 1 mmol/L PMSF (phenyl methyl sulfonyl fluoride)] for 15 min on ice followed by centrifugation at 13,000 g for 15 min. The protein concentration of the supernatant was measured by using the BCA reagents (Pierce, Rockfold, IL, USA). Protein (30 μg) was separated by running through 8 % SDS-PAGE gel and transferred to the PVDF membrane (Gelman Laboratory, Ann Arbor, MI, USA). The blots were blocked with 5 % nonfat dry milk PBST buffer (PBS containing 0.1 % Tween-20) for 1 h at room temperature. The membranes were incubated for 2 h at room temperature with 1:1000 dilution of one of the antibodies of P-STAT3, P-STAT1, P-JAK2, P-AKT, P-ERK, PIAS, SQSTM1/p62, (Cell Signaling Technology, Beverly, MA, USA) or STAT3 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Equal lane loading was assured using actin (Sigma Chemical Co., St. Louis, MO). The blots were rinsed three times with PBST buffer for 10 min each. Washed blots were treated with 1:5000 dilution of the horseradish peroxidase conjugated-secondary antibody (Pierce Biotechnology, Rockford, IL, USA) for 1 h and washed again three times with PBST buffer. The transferred proteins were visualized with an enhanced chemiluminescence detection kit (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Immunoprecipitation

MCF10A-RAS cells were treated with biotinylated SH48 for 24 h, and cells were lysed in 250 mmol/L sucrose, 50 mmol/L Tris-HCl (pH 8.0), 25 mmol/L KCl, 5 mmol/L MgCl2, 1 mmol/L EDTA, 2 mmol/L NaF, 2 mmol/L sodium orthovanadate and 1mmol/L PMSF (phenyl methyl sulfonyl fluoride). Total protein (500 μg) was subjected to immunoprecipitation by shaking with p62 primary antibody at 4°C for 12 h. followed by the addition of protein A/G-agarose bead suspension (25 % slurry, 20 mL) and additional shaking for 2 h at 4°C. After centrifugation at 10,000 rpm for 1 minute, immunoprecipitated beads were collected by discarding the supernatant and washed with cell lysis buffer. The immunoprecipitate was then resuspended in 8 μL of 6x SDS electrophoresis sample buffer and boiled for 5 minutes. Supernatant (48 μL) from each sample was collected by centrifugation and loaded on SDS-polyacrylamide gel. The incorporation of p62 into immunoprecipitated proteins was visualized by use of STAT3 antibody. In advanced research, SH48 show most potent STAT3 inhibitory and antiproliferative activities among the deguelin anaogues tested.

Mass spectrometer analysis

Recombinant STAT3 was purchased from Active motif. Ten μl of recombinant STAT3 protein was mixed well with 0.5 mM SH48 and 40 μl optimized buffer AM1 (20 mM Tris-Cl (pH 8), 20 % glycerol, 100 mM KCl, 1 mM DTT and 0.2 mM EDTA). The mixtures were then subjected to mass spectrometry.

RESULTS

Concentration dependent inhibition of phosphorylation of STAT3 by SH48 in MCF10A-RAS cells.

SH48 that has modified deguelin chemical structure was prepared (Fig. 1). Protein expression of P-STAT3, STAT3, P-STAT1, P-JAk2, and actin was tested by Western blot to determine the effects of SH48 on STAT3 signaling in *ras*-transformed human mammary epithelial MCF10A-RAS cells. P-STAT3 protein expression is decreased depending on SH48 concentrations. Moreover, STAT3 and P-JAk2 protein expression in MCF10A-RAS cells was decreased by 10 μM of SH48. SH48 did not affect P-STAT1, P-AKT and P-ERK protein expression in MCF10A-RAS cells (Fig. 2).

Time dependent inhibition of phosphorylation of STAT3 after SH48 treatment in MCF10A-RAS cells.

To find out elapse time that SH48 effectively suppresses phosphorylation of STAT3 in MCF10A-RAS cells, I tested the protein expression of P-STAT3 in MCF10A-RAS cell after 6 h, 12 h, 18 h and 24 h treatment of SH48 and DMSO (control). P-STAT3 is decreased in a time dependent manner but STAT3 and P-JAk2 protein expression was not affected. In addition PIAS expression was increased 6 h but decreased after that hour (Fig. 3).

The α,β unsaturated carbonyl moiety is essential for inhibition of STAT3 phosphorylation by SH48

MCF10A-RAS cells were treated 5 μM of SH48, SH42, SH43, SSI-1204 or SSI-1205 for 24h. SH42 has substitution in a double bond with hydrogen in comparison with SH48. SH43 has a single bond, while SSI-1204 has substitution in the methyl group. SSI-1205 has substitution in the carbonyl group and some addition in a benzene ring (Fig. 4A). Then, protein expression of P-STAT3, STAT3 and actin was measured by Western blot analysis. P-STAT3 expression was decreased by SH48 and SSI-1204 treatment. Total STAT3 expression was not affected by SH48 treatment (Fig. 4B).

SH48 binds to C328, C367 and C687 residues of STAT3

computer structure modeling program shows cysteine 259 residue of STAT3 as a putative target for SH48 binding. However, mass spectrometric analysis indicates interaction between SH48 and C328, C367 or C687 residue of STAT3. Their peripheral amino sequences are QP**C**MPMHPDRPLVIK, (Fig. 7-1), IKV**C***IDKDSGDVAALRGSR, (Fig. 7-2) and Y**C**RPESQEHPEADPGSAAPYLK (Fig. 7-3), respectively. Among those sites, C328 and C367 belong to the DNA binding domain, and C687 is located in the SH2 domain of STAT3 (Fig. 5).

SH48 induces cell death through accumulation of p62.

In a previous study, the induction of autophagy markers LC3-II and p62 was determined in MCF10A-RAS cells. Their expression was increased by SH48 and autophagic cell death was also increased in a concentration dependent manner. So I checked dependence of p62 on SH48 induced autophagy using a MTT assay. Transient knockdown of p62 abolished SH48- induced MCF10A-RAS cell death (Fig. 8).

p62 and STAT3 interact each other, and SH48 inhibits their binding.

In an attempt to determine the association between inhibition of STAT3 signaling and induction of autophagy by SH48, I examined first interaction between p62 and STAT3 by immunoprecipitation. As shown in Fig. 9, I found novel binding between STAT3 and p62 in MCF10A-RAS cells. SH48 treatment suppressed this interaction in a concentration-dependent manner. Moreover, accumulation of p62 was detected in total cell extract. Treatment of the thiol reducing agent DTT abolish its accumulation (Fig. 10).

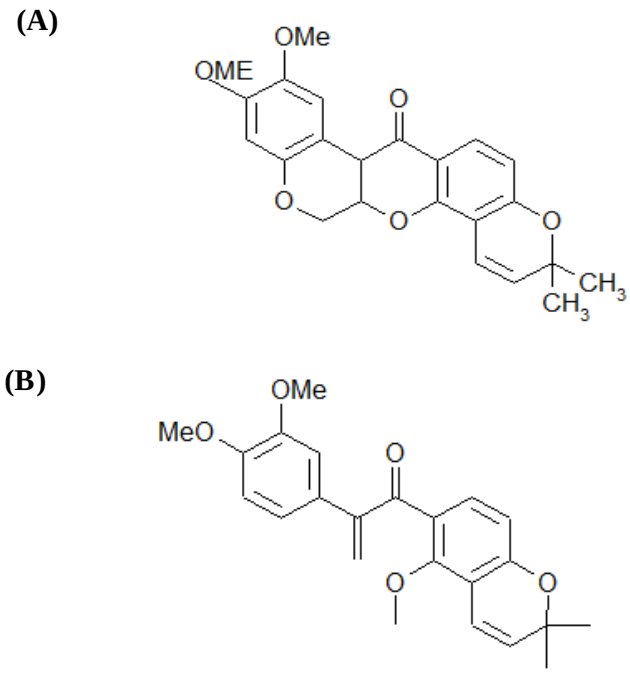


Figure. 1. Structural formulae of deguelin and its analogue SH48.

(A) The chemical structure of deguelin (C23H22O6). The IUPAC name is (7aS,13aS)-13,13a-dihydro-9,10-dimethoxy-3,3-dimethyl-3H bis[1]benzopyrano[3,4-b:6',5'-3]pyran-7(7aH)-one. (B) Structural formula of analogue SH48.

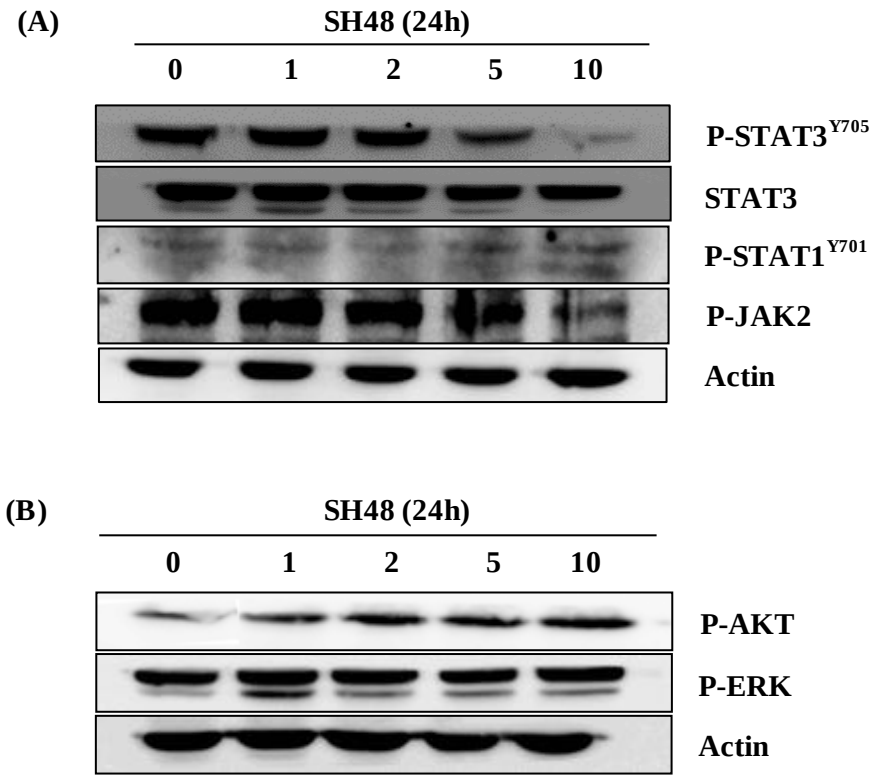


Figure. 2. Effects of the SH48 on expression of P-STAT3, STAT3, P-STAT1, P-JAK2, P-AKT and P-ERK in MCF10A-RAS cells. (A) MCF10A-RAS cells were treated with indicated concentrations of SH48 for 24 h, and expression of P-STAT3, STAT3, P-STAT1 and P-JAK2 was measured by Western blot analysis. (B) MCF10A-RAS cells were treated with indicated concentrations of SH48 for 24 h, and expression of P-AKT and P-ERK was measured by Western blot analysis. Actin was measured for the confirmation of equal amount of protein loaded.

II

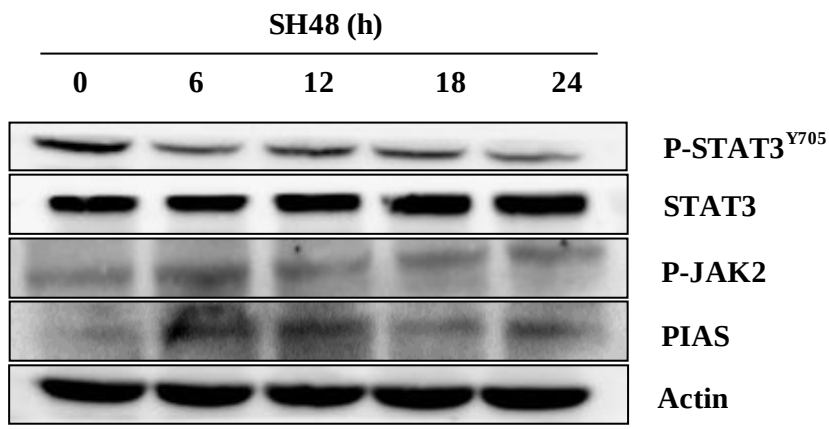


Figure. 3. Effects of SH48 on expression of P-STAT3, STAT3, P-JAK2 and PIAS in MCF10A-RAS cells. MCF10A-RAS cells were treated with 10 μM of SH48 for indicated time periods and expression of P-STAT3, STAT3, P-JAK2 and PIAS was measured by Western blot analysis. Actin was measured for the confirmation of equal amount of protein loaded.

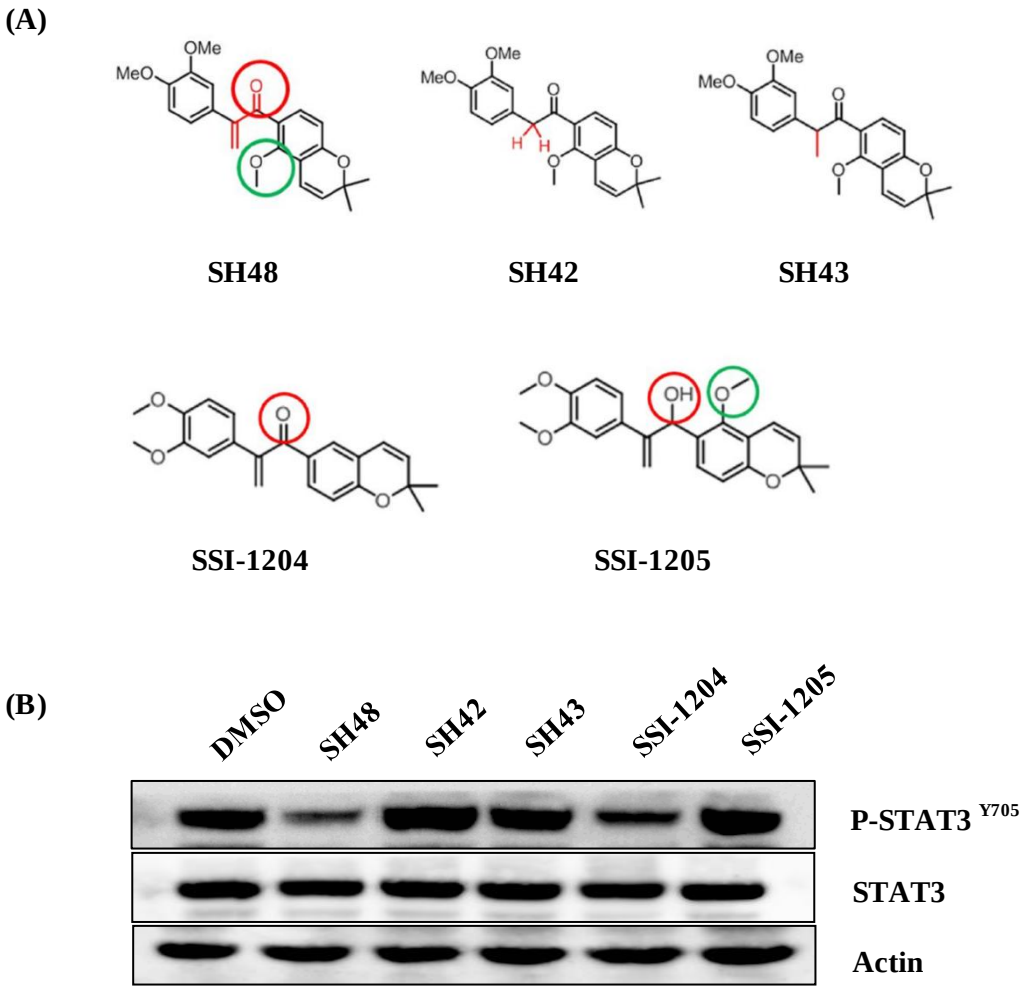


Figure. 4. The α,β unsaturated carbonyl moiety is essential for inhibition of STAT3 phosphorylation by SH48. (A) Structural formulae of deguelin analogues SH42, SH43, SH48, SSI-1204 and SSI-1205. (B) MCF10A-RAS cells were treated with 5 μM of SH48 SH42, SH43, SSI-1204 and SSI-1205 for 24 h, and expression of P-STAT3 and STAT3 was measured by Western blot analysis. Actin was measured for the confirmation of equal amount of protein loaded.

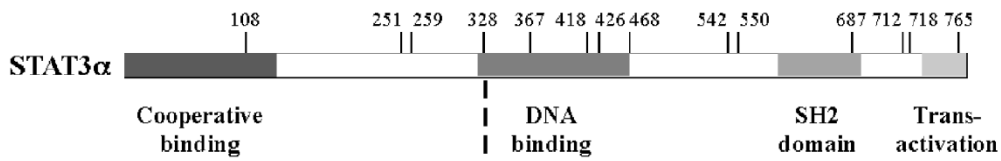


Figure. 5. Cysteine residues of STAT3. A STAT3 dimer formed by inter-chain disulphide bridging during oxidative stress. Adopted from *Biochem. Biophys. Res. Commun*, 24;322(3):1005-11 (2004)

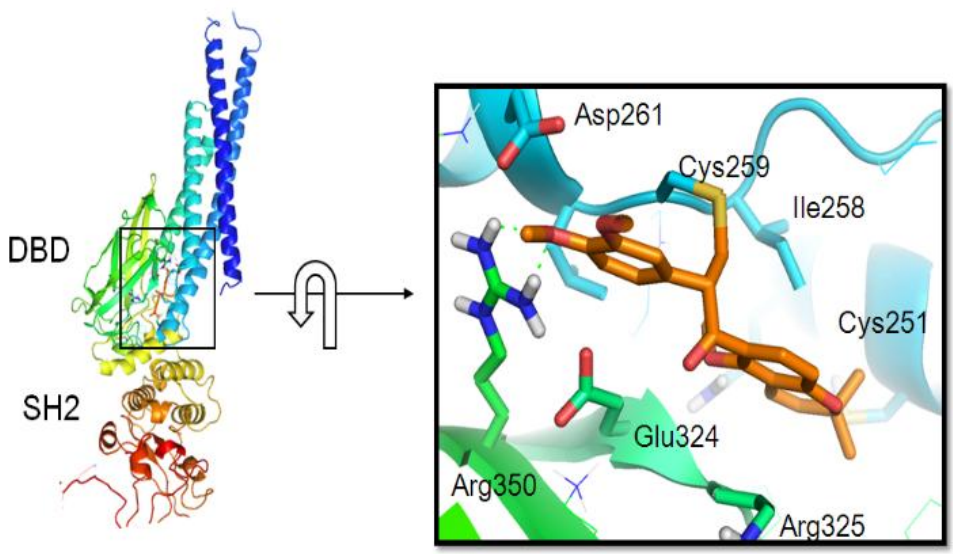


Figure. 6. Putative interaction between the 259 cysteine residue of STAT3 and SH48 determined by the computer modeling

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	114.09135	57.54931	I			16
2	242.18632	121.59680	K	1969.99406	985.50067	15
3	341.25474	171.13101	V	1841.89909	921.45318	14
4	824.42633	412.71680	C-SH48	1742.83067	871.91897	13
5	937.51040	469.25884	I	1259.65908	630.33318	12
6	1052.53735	526.77231	D	1146.57501	573.79114	11
7	1180.63232	590.81980	K	1031.54806	516.27767	10
8	1295.65927	648.33327	D	903.45309	452.23018	9
9	1382.69130	691.84929	S	788.42614	394.71671	8
10	1439.71277	720.36002	G	701.39411	351.20069	7
11	1554.73972	777.87350	D	644.37264	322.68996	6
12	1653.80814	827.40771	V	529.34569	265.17648	5
13	1724.84526	862.92627	A	430.27727	215.64227	4
14	1795.88238	898.44483	A	359.24015	180.12371	3
15	1908.96645	954.98686	L	288.20303	144.60515	2
16			R	175.11896	88.06312	1

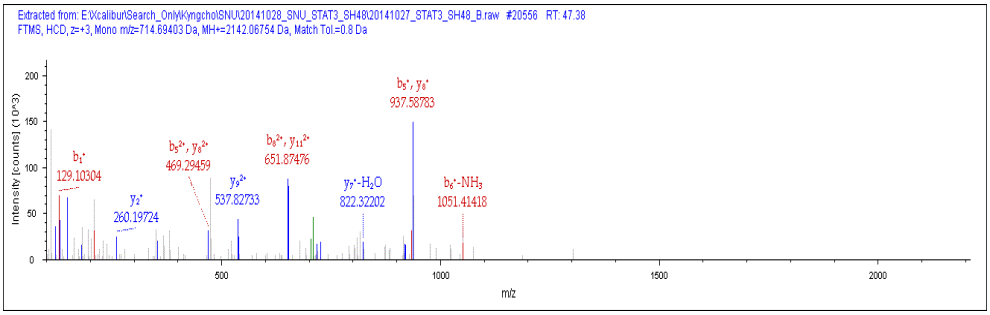


Figure. 7-1. SH48 binds to C328 residue of STAT3. Recombinant STAT3 was purchased from Active motif. Ten µl of recombinant STAT3 protein was mixed well with 0.5 mM SH48 and 40 µl optimized buffer AM1 (20 mM Tris-Cl (pH 8), 20% glycerol, 100 mM KCl, 1 mM DTT and 0.2 mM EDTA). The mixture was then subjected to mass spectrometry. SH48 binds to C328 site. Peripheral amino sequence is QPCMPMHPDRPLVIK.

#1	b^{+}	b^{2+}	b^{3+}	Seq.	y^{+}	y^{2+}	y^{3+}	#2
1	129.06586	65.03657	43.69347	Q				15
2	226.11863	113.56295	76.04439	P	2014.00002	1007.50365	672.00486	14
3	709.29022	355.14875	237.10159	C-SH48	1916.94725	958.97726	639.65393	13
4	840.33072	420.66900	280.78176	M	1433.77566	717.39147	478.59674	12
5	937.38349	469.19538	313.13268	P	1302.73516	651.87122	434.91657	11
6	1068.42399	534.71563	356.81285	M	1205.68239	603.34483	402.56565	10
7	1205.48290	603.24509	402.49915	H	1074.64189	537.82458	358.88548	9
8	1302.53567	651.77147	434.85007	P	937.58298	469.29513	313.19918	8
9	1417.56262	709.28495	473.19239	D	840.53021	420.76874	280.84825	7
10	1573.66374	787.33551	525.22610	R	725.50326	363.25527	242.50594	6
11	1670.71651	835.86189	557.57702	P	569.40214	285.20471	190.47223	5
12	1783.80058	892.40393	595.27171	L	472.34937	236.67832	158.12131	4
13	1882.86900	941.93814	628.29452	V	359.26530	180.13629	120.42662	3
14	1995.95307	998.48017	665.98921	I	260.19688	130.60208	87.40381	2
15				K	147.11281	74.06004	49.70912	1

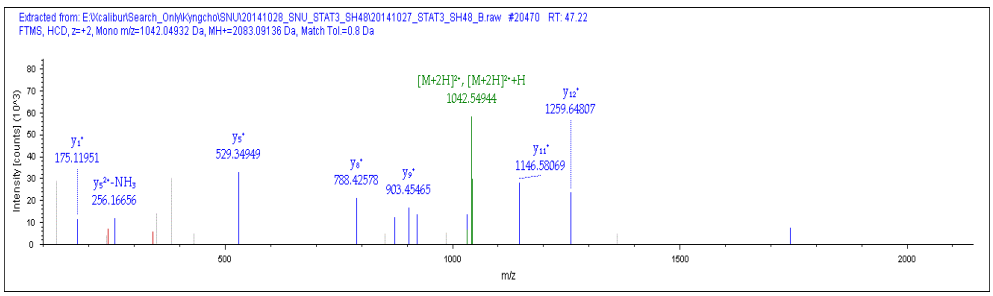


Figure. 7-2. SH48 binds to C367 residue of STAT3. Recombinant STAT3 was purchased from Active motif. Ten µl of recombinant STAT3 protein was mixed well with 0.5 mM SH48 and 40 µl optimized buffer AM1 (20 mM Tris-Cl (pH 8), 20% glycerol, 100 mM KCl, 1 mM DTT and 0.2 mM EDTA). The mixture was then subjected to mass spectrometry. SH48 binds to the C367 site. Peripheral amino sequence is IKVC*IDKDSGDVAALRGSR. *SH48 (+380.1624) modification.

#1	b^{+}	b^{2+}	b^{3+}	Seq.	y^{+}	y^{2+}	y^{3+}	#2
1	100.07570	50.54149	34.03008	V				14
2	583.24729	292.12728	195.08728	C-SH48	1742.83067	871.91897	581.61507	13
3	696.33136	348.66932	232.78197	I	1259.65908	630.33318	420.55788	12
4	811.35831	406.18279	271.12429	D	1146.57501	573.79114	382.86319	11
5	939.45328	470.23028	313.82261	K	1031.54806	516.27767	344.52087	10
6	1054.48023	527.74375	352.16493	D	903.45309	452.23018	301.82255	9
7	1141.51226	571.25977	381.17560	S	788.42614	394.71671	263.48023	8
8	1198.53373	599.77050	400.18276	G	701.39411	351.20069	234.46955	7
9	1313.56068	657.28398	438.52508	D	644.37264	322.68996	215.46240	6
10	1412.62910	706.81819	471.54788	V	529.34569	265.17648	177.12008	5
11	1483.66622	742.33675	495.22692	A	430.27727	215.64227	144.09727	4
12	1554.70334	777.85531	518.90596	A	359.24015	180.12371	120.41823	3
13	1667.78741	834.39734	556.60065	L	288.20303	144.60515	96.73919	2
14				R	175.11896	88.06312	59.04450	1

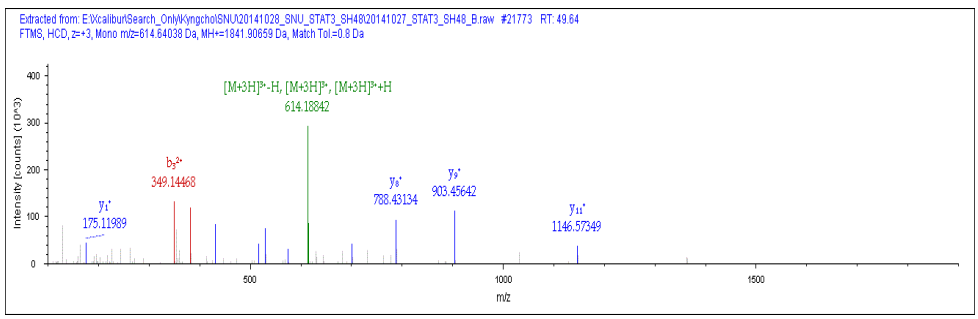


Figure. 7-3. SH48 binds to C367 residue of STAT3. Recombinant STAT3 was purchased from Active motif. Ten µl of recombinant STAT3 protein was mixed well with 0.5 mM SH48 and 40 µl optimized buffer AM1 (20 mM Tris-Cl (pH 8), 20% glycerol, 100 mM KCl, 1 mM DTT and 0.2 mM EDTA). The mixture was then subjected to mass spectrometry. SH48 binds to C367 site. Peripheral amino sequence is VCIDKDSGDVAALR.

#1	b^{+}	b^{2+}	Seq.	y^{+}	y^{2+}	#2
1	164.07060	82.53894	Y			22
2	647.24219	324.12473	C-SH48	2662.21312	1331.61020	21
3	803.34331	402.17529	R	2179.04153	1090.02440	20
4	900.39608	450.70168	P	2022.94041	1011.97384	19
5	1029.43868	515.22298	E	1925.88764	963.44746	18
6	1116.47071	558.73899	S	1796.84504	898.92616	17
7	1244.52929	622.76828	Q	1709.81301	855.41014	16
8	1373.57189	687.28958	E	1581.75443	791.38085	15
9	1510.63080	755.81904	H	1452.71183	726.85955	14
10	1607.68357	804.34542	P	1315.65292	658.33010	13
11	1736.72617	868.86672	E	1218.60015	609.80371	12
12	1807.76329	904.38528	A	1089.55755	545.28241	11
13	1922.79024	961.89876	D	1018.52043	509.76385	10
14	2019.84301	1010.42514	P	903.49348	452.25038	9
15	2076.86448	1038.93588	G	806.44071	403.72399	8
16	2163.89651	1082.45189	S	749.41924	375.21326	7
17	2234.93363	1117.97045	A	662.38721	331.69724	6
18	2305.97075	1153.48901	A	591.35009	296.17868	5
19	2403.02352	1202.01540	P	520.31297	260.66012	4
20	2566.08684	1283.54706	Y	423.26020	212.13374	3
21	2679.17091	1340.08909	L	260.19688	130.60208	2
22			K	147.11281	74.06004	1

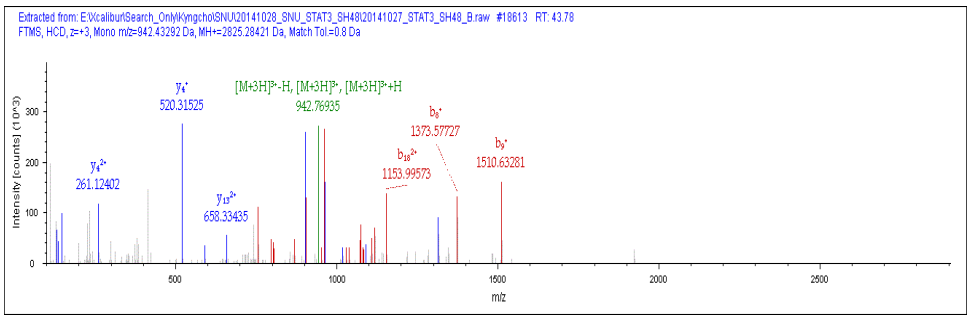


Figure. 7-4. SH48 binds to C687 residues of STAT3. Recombinant STAT3 was purchased from Active motif. Ten µl of recombinant STAT3 protein was mixed well with 0.5 mM SH48 and 40 µl optimized buffer AM1 (20 mM Tris-Cl (pH 8), 20% glycerol, 100 mM KCl, 1 mM DTT and 0.2 mM EDTA). The mixture was then subjected to mass spectrometry. SH48 binds to C687 site. Peripheral amino sequence is YCRPESQEHPEADPGSAAPYLK.

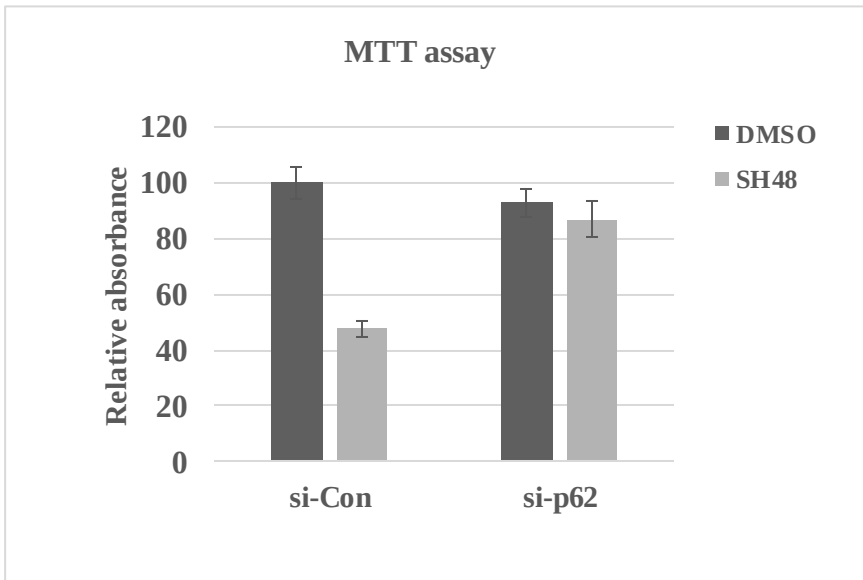


Figure. 8. SH48-induced autophagy is mediated by p62. MCF10A-RAS cells were transiently transfected with si-p62 or si-control for 6 h, followed by treatment with 10 μM of SH48 for 24 h, and viability of MCF10A cells was measured by the MTT assay.

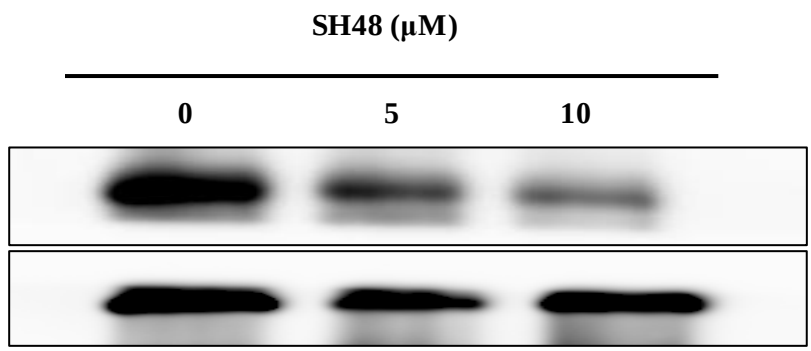
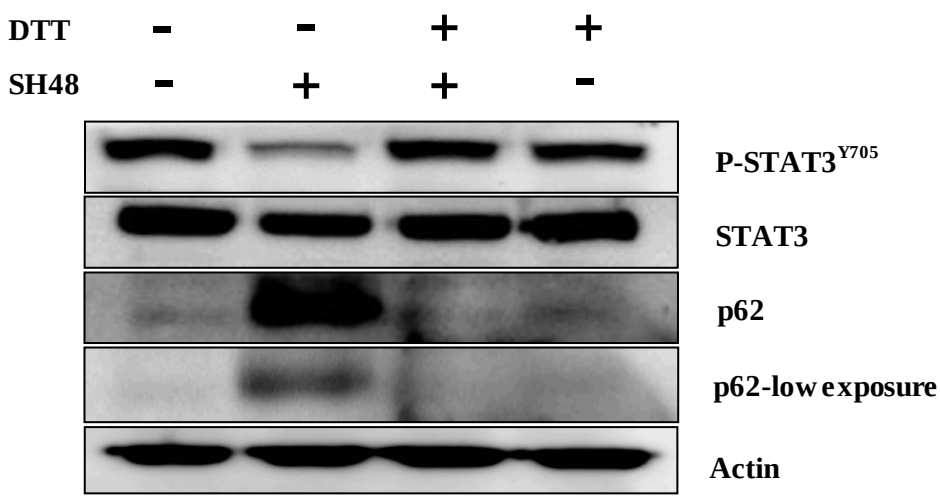


Figure. 9. p62 and STAT3 interact each other, and SH48 inhibits their binding. (A) MCF10A-RAS cells were treated with indicated concentrations of SH48 for 24 h. Protein lysates were immunoprecipitated with STAT3 antibody and analyzed by Western blot analysis with anti-p62 antibody.



DTT (dithiothreitol): treducing agent

Figure. 10. p62 protein expression is associated with STAT3 inhibition by SH48. MCF10A-RAS cells were treated with 5 μM of SH48 for 24 h, and DTT was treated for 4 h. The expression of P-STAT3, STAT3, and p62 was measured by Western blot analysis.

DISCUSSION

Constitutively activated STAT3 is found frequently in a wide variety of human tumors [33]. STAT3 regulates the expression of various genes involved in proliferation, apoptosis, angiogenesis, invasion and metastasis by regulating expression of various genes and actively participates in tumor formation and progression [34]. Therefore, inhibition of STAT3 provides a rational strategy to block cancer development [35].

Currently two major strategies are used to inhibit the STAT3 signaling pathway. At first, direct inhibition of STAT3 protein with inhibitors targeting one of three structural domains of STAT3, namely SH2 domain, DNA binding domain and N-terminal domain, which suppresses processes related to STAT3 signaling and its functional role by blocking phosphorylation, dimerization, nuclear translocation and DNA binding or by indirectly blocking the upstream regulators of STAT3 pathway [35].

The inhibition of aberrant STAT3 activity by genetic or pharmacological approaches include small molecular weight inhibitors and compounds, protein inhibitors, dominant-negative STAT3 mutants, antisense RNA and interference oligonucleotides (RNAi). Natural products and their synthetic analogues have been known as important sources in developing STAT3 inhibitors [36]. For instance, curcumin, betulinic acid, capsaicin, caffeic acid, celastro, cucurbitacins,

guggulsterone, diosgenin and honokiol are known as natural STAT3 inhibitors [37]. In a similar context, a series of analogues of deguelin were synthesized to develop novel STAT3 inhibitors. Among them, an electrophilic analogue SH48 bearing an α,β unsaturated carbonyl group was found to inhibit phosphorylation, dimerization, nuclear translocation and transcriptional activity of STAT3. However, the mechanisms underlying SH48-induced STAT3 inhibition were not fully elucidated. It is possible that SH48 may also inhibit STAT3 indirectly. Therefore, I intended to conduct an experiment to confirm previous study of STAT3 inhibition by SH48 and determined the effects of SH48 on expression of P-STAT3, P-STAT1, P-JAK2, P-AKT and P-ERK in MCF10A-RAS cells. The carbonyl group of SH48 was postulated to bind thiol groups of any cysteine residues in various signaling proteins. SH48 is also anticipated to affect HSP90 client proteins (P-STAT3, P-JAK2, P-AKT and P-ERK). However, only P-STAT3 was affected by SH48. Additional mechanistic studies are needed to elucidate its specific influence on STAT3 phosphorylation.The critical functional moiety of SH48 involved in its inhibition of STAT3 signaling was assessed by comparison with SH42, SH43, SH48, SSI-1204 and SSI-1205 analogues. As a results, it was found that the α,β unsaturated carbonyl moiety is essential for inhibition of STAT3 phosphorylation by SH48. SSI-1205 showed a similar inhibitory effect on phosphorylation of STAT3. However, it was excluded because it did not show significant inhibitory effect on viability of MCF10A-RAS cells. The methyl group of SH48 may also be important in its inhibition of STAT3 phosporylation.

Then, specific interaction between SH48 and the cysteine 259 residue of STAT3 was anticipated based on the fact that this amino acid is located in the short loop between helices a2 and a3 of the amino-terminal four-helix bundle of STAT3 which lies against the core b-barrel of the monomer. Therefore, cysteine 259 is exposed in the surface of tertiary structure of STAT3 and considered to be prone to disulfide bridge formation upon oxidative stress [38].

The possible interaction between SH48 and Cys259 of STAT3 was assessed by mass spectral analysis. However, mass-spectral data indicate that the binding sites are C328, C367 or C687 residues of STAT3. Although the function of these cysteine residues of STAT3 was not reported yet, the binding of SH48 to STAT3 can be influenced by their localization in STAT3. C328 or C367 are located in the DNA binding domain and C687 exists in the SH2 domain of STAT3. Each site is likely to be related to transcriptional activity as well as phosphorylation and dimerization of STAT3.

Inhibition of STAT3 signal was reported to cause cell death [39]. In this study STAT3 inhibition by SH48 caused reduction of cell viability. However, SH48 did not induce apoptosis as determined by cleavage of PARP and reduction in the levels of the anti-apoptotic protein Bcl-xL. Therefore, SH48-induced cell death is unlikely to be mediated by apoptosis. Notably, protein expression of LC3-II and p62 which are autophagy markers was increased. In addition, increased autolysosome formation was detected in an experiment using a fluorescent

acidotropic probe LysoTracker Red.

Many drugs are known to block the activation of the transcription factors by inducing autophagy [40]. Therefore, I conducted additional experiments to confirm the mode of SH48-induced MCF10A-RAS cell death with special focus on autophagy and tried to determine the association between inhibition of STAT3 signaling and induction of autophagy by SH48. SH48 induced LC3-I to LC3-II conversion and p62 protein expression. I found a noble binding between STAT3 and p62 and this interaction was inhibited by SH48. Therefore, the interaction between SH48 and STAT3 is speculated to facilitate the accumulation of free p62, which may account for autophagy induction by SH48.

As mentioned earlier, p62 acts as a signaling hub through its ability to recruit and oligomerize important signaling molecules in cytosolic speckles to control cell survival and apoptosis [41]. p62 is also considered as a cargo adapter for ubiquitinated proteins that can be degraded by autophagy [42].

In the cancer cell, p62 acts either as a pro-oncogene or as a tumor suppressor protein. Based on the presence of multiple interacting domains in p62/SQSTM1, this protein is also able to positively modulate either the NFκB pathway by binding to the IKK activator TRAF6 or the mTOR pathway by interacting with Raptor [43]. In contrast to its pro-oncogenic activity, p62 can also exert tumor suppressive activity by promoting inactivation of the Wnt pathway via an autophagic process [44]. Therefore, p62 is considered as a central player in the life and death decisions

of the cancer cell.

The existence of autophagic cell death in mammalian cells also remains controversial. Auophagy can be upregulated or suppressed by cancer therapeutics, and upregulation of autophagy in cancer therapies can be either pro-survival or pro-death for tumor cells [40].

In summary, the synthetic deguelin analogue SH48 inhibits phosphorylation, DNA-binding activity, and transcriptional activity of STAT3 possibly by direct interaction with the C328, C367 or C687 cysteine residue of STAT3 in MCF10A-RAS cells. Its direct binding with STAT3 induces autophagy through accumulation of p62.

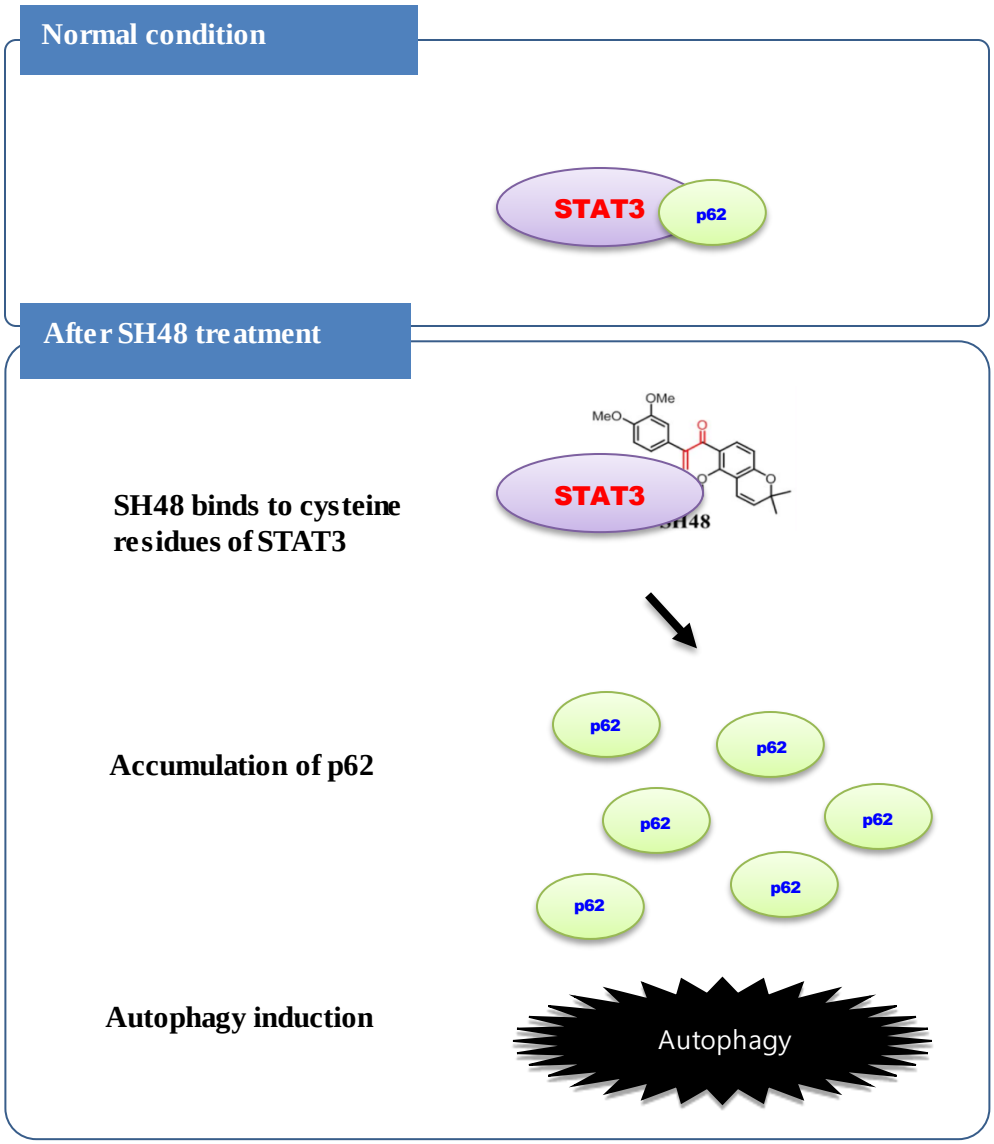


Figure. 11. Proposed mechanism for p62 protein accumulation and induction of autophagy as a consequence of STAT3 inhibition by SH48. p62 binds to STAT3 in normal condition**.** SH48 binds to cysteine residues of STAT3. SH48 inhibits interaction between p62 and STAT3 and may facilitate accumulation of p62.

REFERENCES

1. Yoshitani SI, Tanaka T, Kohno H and Takashima S. (2001) Chemoprevention of azoxymethane-induced rat colon carcinogenesis by dietary capsaicin and rotenone. *Int J Oncol*., 19: 929-939.

2. Thamilselvan V, Menon M and Thamilselvan S. (2011) Anticancer efficacy of deguelin in human prostate cancer cells targeting glycogen synthase kinase-3 beta/beta-catenin pathway. *Int J Cancer*., 129: 2916-2927.

3. Matsuda H1, Yoshida K, Miyagawa K, Asao Y, Takayama S, Nakashima S, Xu F, Yoshikawa M. (2007) Rotenoids and flavonoids with anti-invasion of HT1080, anti-proliferation of U937, and differentiation-inducing activity in HL-60 from Erycibe expansa. *Bioorg Med Chem.*, 15: 1539-1546.

4. Zhengguang li, Jun Wu, Changping Wu, Jingting Jiang, Xiao Zheng, Bin Xu, and Min Li. (2012) Deguelin, a natural rotenoid, inhibits mouse myeloma cell growth in vitro via induction of apoptosis. *Oncol Lett.*, 4(4): 677–681.

5. Ho-Young Lee, Young-Ah Suh, Jerome W. Kosmeder, John M. Pezzuto, Waun Ki Hong, and Jonathan M. Kurie. (2004) Deguelin-Induced Inhibition of Cyclooxygenase-2 Expression in Human Bronchial Epithelial Cells. *Clinical Cancer Research.*, Vol. 10, 1074–1079.

6. Yang Y-l, Ji C, Bi Z-g, Lu C-c, Wang R, et al. (2013) Deguelin induces both apoptosis and autophagy in cultured head and neck squamous cell carcinoma cells. *PLoS ONE.*, 8(1): e54736.

7. Oh SH1, Woo JK, Yazici YD, Myers JN, Kim WY, Jin Q, Hong SS, Park

HJ, Suh YG, Kim KW, Hong WK, Lee HY. (2007) Structural Basis for Depletion of Heat Shock Protein 90 Client Proteins by Deguelin. *J Natl Cancer Inst*., 20;99(12):949-61.

8. Shigeki Ito, MD PhD1, Tatsuo Oyake, MD PhD1,*, Kazunori Murai, MD1,* and Yoji Ishida, MD PhD1. (2007) Deguelin, a Novel Hsp90 Inhibitor, Suppresses STAT3 and STAT5 Activity and Induces Apoptosis in the Human T-Cell Leukemia Virus Type 1-Transformed Cells. *Blood (ASH Annual Meeting Abstracts).*, 110-1387.

9. Vrana JA1, Boggs N, Currie HN, Boyd J. (2013) Amelioration of an undesired action of deguelin. *Toxicon*., 74:83-91.

10. Hua Yu, Heehyoung Lee, Andreas Herrmann, Ralf Buettner & Richard Jove. (2014) Revisiting STAT3 signalling in cancer: new and unexpected biological functions. *Nature Reviews Cancer* 14., 736–746.

11. Darnell JE Jr, Kerr IM, Stark GR. (1994) Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science*., 264:1415–21.

12. Yu H, Pardoll D, Jove R. (2009) STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat Rev Cancer*.,798–809.

13. Zhang X1, Sun Y, Pireddu R, Yang H, Urlam MK, Lawrence HR, Guida WC, Lawrence NJ, Sebti SM. (2013) A Novel Inhibitor of STAT3 Homodimerization Selectively Suppresses STAT3 Activity and Malignant Transformation. *Cancer Res*., 15;73(6):1922-33.

14. Subramaniam A1, Shanmugam MK, Perumal E, Li F, Nachiyappan A, Dai X, Swamy SN, Ahn KS, Kumar AP, Tan BK, Hui KM, Sethi G. (2013) Potential role of signal transducer and activator of transcription (STAT) 3 signaling pathway in inflammation, survival, proliferation and invasion of

hepatocellular carcinoma. *Biochim Biophys Acta*., 1835(1):46-60.

15. Gabriella Miklossy, Tyvette S. Hilliard & James Turkson. (2013) Therapeutic modulators of STAT signalling for human diseases. *Nature Reviews Drug Discovery*., 12, 613629.

16. Zhineng J. Yang, Cheng E. Chee, Shengbing Huang, and Frank A. (2011) Sinicrope: The Role of Autophagy in Cancer: Therapeutic Implications, *Mol Cancer Theraphy.*, MCT-11-0047 *10;* 1533.

17. Yenniffer Á valos, Jimena Canales, Roberto Bravo-Sagua, Alfredo Criollo, Sergio Lavandero, and Andrew F. G. Quest1. (2014) Tumor Suppression and Promotion by Autophagy. *BioMed Research International*., Article ID 603980.

18. Cuervo AM, Dice JF. (1996) A receptor for the selective uptake and degradation of proteins by lysosomes. *Science*., 273:501-3.

19. Jayanta Debnath, Eric H. Baehrecke, Guido KroemerDoes. (2005) Autophagy Contribute to Cell Death?. *Autophagy*., 1:2, 66-74.

20. Pattingre S, Espert L, Biard-Piechaczyk M, Codogno P. (2008) Regulation of macroautophagy by mTOR and Beclin 1 complexes. *Biochimien*., 90:313–23.

21. Shen S1, Niso-Santano M, Adjemian S, Takehara T, Malik SA, Minoux H, Souquere S, Mariño G, Lachkar S, Senovilla L, Galluzzi L, Kepp O, Pierron G, Maiuri MC, Hikita H, Kroemer R, Kroemer G. (2012) Cytoplasmic STAT3 represses autophagy by inhibiting PKR activity. *Mol Cell*., 14;48(5):667-80.

22. Gong J, Muñoz AR, Chan D, Ghosh R, Kumar AP. (2014) STAT3 down regulates LC3 to inhibit autophagy and pancreatic cancer cell growth. *Oncotarget*., 15;5(9):2529-41.

23. K Castillo, V Valenzuela, S Matus, M Nassif, M Oñate, Y Fuentealba, G Encina, T Irrazabal, G Parsons, F A Court, B L Schneider, D Armentano and C Hetz. (2013) Measurement of autophagy flux in the nervous system in vivo. *Cell Death and Disease.*, 4, e917.

24. Sharon Manley, Jessica A. Williams, and Wen-Xing Ding. (2014) The Role of p62/SQSTM1 in Liver Physiology and Pathogenesis. *Exp Biol Med.*, 238(5): 525–538.

25. Geisler, S., Holmstrom, K.M., Skujat, D., Fiesel, F.C., Rothfuss, O.C., Kahle, P.J.and Springer, W. (2010) PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat. Cell Biol.*, 12, 119–131.

26. Pohl, C. and Jentsch, S. (2009) Midbody ring disposal by autophagy is a postabscission event of cytokinesis. *Nat. Cell Biol.*, 11, 65–70.

27. Kim, P.K., Hailey, D.W., Mullen, R.T. and Lippincott-Schwartz, J. (2008) Ubiquitin signals autophagic degradation of cytosolic proteins and peroxisomes. *Proc. Natl.* Acad. Sci. USA 105, 20567–20574.

28. Yoshikawa, Y. et al. (2009) Listeria monocytogenes ActA-mediated escape from autophagic recognition. *Nat. Cell Biol.*, 11, 1233–12340.

29. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. (2008) Autophagy fights disease through cellular self-digestion. *Nature.*, 451:1069–1075.

30. Eileen White. (2012) Deconvoluting the context-dependent role for autophagy in cancer. *Nature Reviews Cancer* 12., 401-410.

31. Bo Liu , Yan Cheng , Qian Liu , Jin-ku Bao and Jin-Ming Yang. (2010) Autophagic pathways as new targets for cancer drug development. *Acta Pharmacologica Sinica.*, 31: 1154–1164.

32. Yang CH, Fan M, Slominski AT, Yue J, Pfeffer LM. (2010) The role of

constitutively activated STAT3 in B16 melanoma cells. *Int J Infereron Cytokine Mediator Res*., 1-7.

33. Haura, E.B.; Turkson, J.Jove, R. (2005) Mechanisms of disease: Insights into the emerging role of signal transducers and activators of transcription in cancer. Nat. *Clin. Pract. Oncol*., 2, 315–324.

34. Ailian Xiong,1 Zhengduo Yang, Yicheng Shen, Jia Zhou, and Qiang Shen1. (2014) Transcription Factor STAT3 as a Novel Molecular Target for Cancer Prevention. *Cancers*., 6(2): 926–957.

35. Peibin Yue, MD PhD and James Turkson, Ph. (2009) Targeting STAT3 in cancer: how successful are we?. *Expert Opin Investig Drugs.*, 18(1): 45–56.

36. Xiaochun Wang Philip J. Crowe David Goldstein Jia-Lin Yang. (2012) STAT3 inhibition, a novel approach to enhancing targeted therapy in human cancers (Review). *Int J Oncol*., 41(4):1181-91.

37. Kodappully Sivaraman Siveena, Sakshi Sikkaa, Rohit Suranaa, Xiaoyun Daia, Jingwen Zhanga, Alan Prem Kumara, Benny K.H. Tana, Gautam Sethia, Anupam Bishayeee. (2014) Targeting the STAT3 signaling pathway in cancer: Role of synthetic and natural inhibitors. *Biochimica et Biophysica Acta (BBA)* - Reviews on Cancer Volume 1845., Issue 2, Pages 136–154.

38. Li L, Shaw PE. (2004) A STAT3 dimer formed by inter-chain disulphide bridging during oxidative stress. *Biochem Biophys Res Commun*. 322(3):1005-11.

39. Kreuzaler PA, Staniszewska AD, Li W, Omidvar N, Kedjouar B, Turkson J, Poli V, Flavell RA, Clarkson RW, Watson CJ. (2011) Stat3 controls lysosomal-mediated cell death in vivo. *Nat Cell Biol*., 13(3):303-9.

40. Liu, Emma Y., and Kevin M. Ryan. (2012) Autophagy and cancer–issues we need to digest. *Journal of cell science.*, 2349-2358.

41. Johansen T, Lamark T. (2011) Selective autophagy mediated by autophagic adapter proteins. *Autophagy.*, 7(3): 279–296.

42. Moscat J, Diaz-Meco MT. (2009) p62 at the crossroads of autophagy, apoptosis, and cancer. *Cell.*, 137(6):1001-4.

43. Alexandre Puissant, Nina Fenouille, and Patrick Auberger. (2012) When autophagy meets cancer through p62/SQSTM. *Cancer Res.*, 397–413.

44. Duran A, Amanchy R, Linares JF, Joshi J, Abu-Baker S, Porollo A, Hansen M, Moscat J, Diaz-Meco MT. (2011) p62 is a key regulator of nutrient sensing in the mTORC1 pathway. *Mol Cell.*, 44:134–146.

국문초록

JAK/STAT 신호전달 체계에서 STAT3 (Signal Transducer and Activator of Transcription 3)는 종양 미세환경 내 악성 세포의 증식과 생존, 침윤, 혈관신생과 만성염증을 조절 하는 중요한 전사인자이다. 기존의 연구에서 새로운 STAT3 저해제 개발을 위해 식물유래 천연 로테노이드 (rotenoid)의 한 종류인 deguelin으로부터 일련의 유도체들을 합성하였으며, 이들 합성 유사체중 SH48이 STAT3의 인산화와 이합체화 (dimerization) 그리고 핵내 이동 및 전사활성을 저해함을 확인 하였으며 또한 p62와 LC3-II 단백질 발현증가를 통한 세포자식 작용 유도를 확인하였다. 이어 본 연구에서는 질량 분석법을 통해 SH48과 STAT3의 328, 367, 687번 시스테인 잔기 간의 직접적인 결합을 확인하였으며 SH48이 p62에 의존적으로 자식작용을 유도함을 확인하였고 STAT3와 p62사이의 직접적 결합을 확인 하였으며 SH48에 의한 이들 결합의 저해와 p62단백질의 발현 증가를 확인 하였다.

따라서 본 연구는 SH48과 STAT3사이의 직접적인 결합에 의해 STAT3활성화와 p62단백질과 STAT3사이의 결합이 억제되고 이를 통한 p62 단백질의 축척이 MCF10A-RAS cell의 세포자식 작용을 유발함을 시사한다.

주요어 (Keywords): Signal transducer and activator of transcription 3 (STAT3), deguelin, SH48, Autophagy, p62/SQSTM1, Atg8/LC3-II.

학번 (Student Number): 2013-21581