의학 석사 학위 논문

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Transcriptional regulation of Ribosomal protein L7a

by

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Abstract

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Ribosomal proteins (RPs) are main components of ribosome catalyzing protein synthesis in the cytoplasm. In addition to synthesizing protein, RPs achieves various cellular processes such as replication, transcription, RNA processing, DNA repair and even inflammation.

Transglutaminase 2 (TGase 2) is Ca^{2+}-dependent enzyme that catalyzes crosslinking, polyamination, and deamidation on glutamine side chains in proteins. TGases 2 activity increases in response to various stresses, and abrupt activation of TGase 2 is associated with various disease conditions such as cataract, fibrosis, neurodegeneration and cancer. Previous study showed that Ribosomal protein L7a (RPL7a) functions as a negative regulator of TGase 2 activity. In this study, we investigated the transcription factors that regulate the expression of RPL7a. Using luciferase assay, we defined active transcription region in RPL7a promoter and identified p53 and TFII-I as putative transcription factors.

Overexpression of p53 decreased RPL7a promoter activity, mRNA and protein level. On the contrary, knockdown of TFII-I by RNAi decreased RPL7a promoter activity, mRNA, and protein level. In addition, both p53 and TFII-I directly bound to each of the putative binding site in RPL7a promoter. Our results indicate that the expression of RPL7a is reciprocally regulated by p53 and TFII-I, suggesting that regulation of RPL7a expression by p53 and TFII-I may play roles in TGase 2 activity-associated diseases.

Keywords: Ribosomal protein L7a; TFII-I; p53; transcriptional regulation

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Introduction

1. Ribosomal proteins

Functional ribosomes consist of two distinct subunits, large and small, and about 70% of the mass of the ribosome consists of RNA known as ribosomal RNA (rRNA), while ribosomal protein (RP) is a third of the ribosome (Jang et al., 2011). In humans, it has been known that males have 80 ribosomal proteins and females have 79 ribosomal proteins, without knowing their function (Warner and Nierras, 1998). The ribosomal proteins are major constituents of ribosomes catalyzing protein synthesis in the cytoplasm (Mager, 1988). They are named according to the subunit of the ribosome to which they belong, with the proteins belonging to the large subunit being designated L1-L44 and those belonging to the small subunit being designated S1-S31.

Many ribosomal proteins, those of the large subunit, including ribosomal protein L7a (RPL7a) consist of a globular surface-exposed RNA-binding domain binding to the rRNA core to stabilize its structure. Though decoding activities and peptide transfer are based on rRNA, ribosomal proteins also play a critical role in the process of protein synthesis (Wool, 1996). In addition to their function in protein synthesis, a number of ribosomal proteins have a special function outside the ribosome involving various cellular processes such as replication, transcription, RNA processing, DNA repair and even

inflammation (Wool, 1996; Yamamoto, 2000). Moreover, recent data have shown that down-regulation of some ribosomal proteins is related to carcinogenesis (Ebert et al., 2008; Vaarala et al., 1998).

1-1. Human ribosomal protein L7a (hRLP7a).

The protein belongs to the L7AE family of ribosomal proteins. This gene is included in the surfeit gene cluster that does not share sequence similarity. This gene rearranges with the trk proto-oncogene to form the chimeric oncogene trk-2h, which encodes an oncoprotein composed of the N terminus of ribosomal protein L7a fused to the receptor tyrosine kinase domain of trk.

60S ribosomal protein L7a (RPL7a), a constituents of the 60S large ribosomal subunit, also plays an important role in stabilizing ribosomes by binding to rRNA (De Falco et al., 1993; Huxley and Fried, 1990). This protein appendage is mobile, interacts with elongation factors and is involved in inducing their GTPase activity (Maguire and Zimmermann, 2001). RPL7a contains two distinct RNA-binding domains that encompass amino acids 52-100 and amino acids 101-161 (Russo et al., 2005). In addition to its function in the ribosome, this protein may also be relevant to cell growth and differentiation by interacting with human thyroid hormone receptor (THR) and retinoic acid receptor (RAR) and in turn inhibiting the activities of the two nuclear hormone receptors (Burris et al., 1995).

According to previous studies, hRPL7a was involved in the control of cellular transformation, tumor growth, aggressiveness and metastasis. For example, hRPL7a was an ethanol-responsive factor in T47D breast cancer cells and the transcription rate of hRPL7a was not a simple stress response, because other stress inducers, such as heat shock, did not have an impact on the expression of hRPL7a. Furthermore, breast cancer cells expressed higher level of hRPL7a than normal mammary epithelial cells. Especially, hRPL7a activates the trk oncogene by contributing an amino-terminal-activating sequence to the receptor kinase domain of trk. Therefore, altering hRPL7a expression may mediate the promoting effects of ethanol on breast cancer development (Zhu et al., 2001). Also, it has been shown that hRPL7a is associated with malignant brain tumor formation (Kroes et al., 2000). It was recently reported that down regulation of ribosomal proteins was associated with cataract formation (Zhang W, 2002).

2. Transcription factor TFII-I

TFII-I is as a biochemical entity capable of binding to and functioning by way of the Initiator element (Inr) in *in vitro* system with general transcription factors (Roy et al., 1991). Aside from its interaction with the Inr element, TFII-I also interacted with an upstream E-box element recognized by helix-loop-helix (HLH) proteins like USF. TFII-I interacted with an E-box individually as

well as cooperatively with USF (Roy et al., 1991; Roy et al., 1997). While binding and function of TFII-I with Inr element has been reported since then both *in vitro* and *in vivo* (Roy, 2007), it does not appear to be a general transcription factor required for all Inr-containing promoters, especially when assayed in *in vitro* transcription systems with highly purified transcription factors.

2-1. Structural properties of TFII-I

TFII-I (the gene symbol is GTF2I in humans and Gtf2i in mice) belongs to a family of transcription factors, composed of three related genes that are closely located in human chromosome 7 (Hinsley et al., 2004). Each member has multiple alternatively spliced isoforms. The best characterized TFII-I has at least four alternatively spliced isoforms in humans (α, β, γ and Δ) (Roy, 2001). A special structural characteristic of TFII-I members is the presence of a repeated domain (Roy, 2001). For instance, TFII-I has six repeats (Fig. 1a) and each contains a putative HLH motif, a protein-protein interaction module present in E-box binding proteins. The most conserved amino acids within these repeats are called the I-repeats (Fig. 1b; Roy et al., 1997). NMR spectroscopic determination of the three-dimensional structure of the fifth repeat in TFII-I revealed that the TFII-I/GFT2I domain has four helices, two antiparallel strands/sheets (between helices 2 and 3 and between helices 3 and 4) and a long loop composed of two β-turns between helices 2 and 3 (Fig. 1c; Doi-Katayama et al., 2007). It is likely that

all other repeats of TFII-I have similar folds (henceforth called the "I-fold") As the conserved residues usually existing in the various repeats are assembled on the hydrophobic core, β-turns and other secondary structural elements (Doi-Katayama et al., 2007).

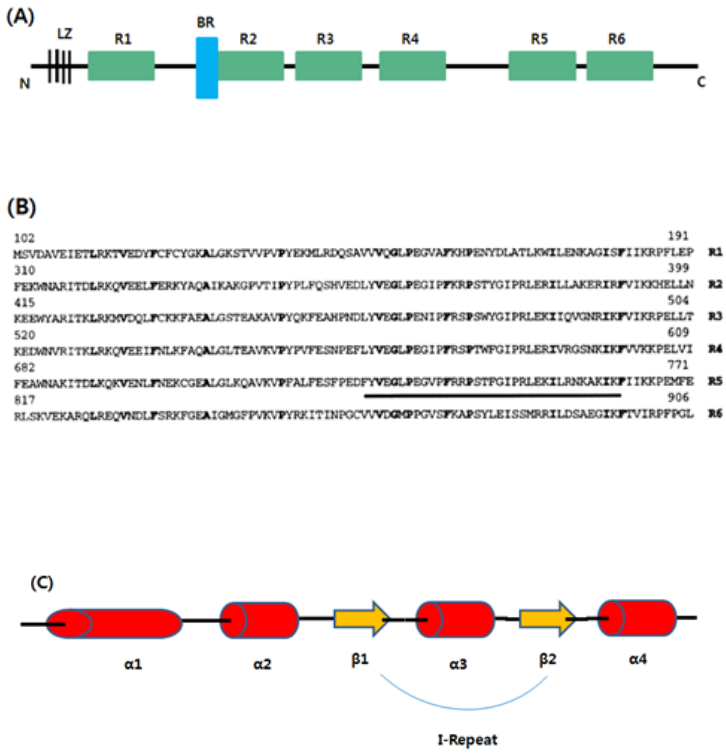


Figure 1. Structural Features of TFII-I (Ananda L. Roy, 2011).

(A) Schematic representation of TFII-I with 6 repeated regions, R1-R6, the N-terminal leucine zipper (LZ) and the basic region (BR)

(B) The sequence of the repeats R1-R6. The most conserved amino acids are indicated in bold and the I-repeat is underlined.

(C) The linear arrangement of the murine repeat 5 (R5). The various amphipathic helices are indicated as α1-4 and the beta sheets are indicated as β1 and β2. This unique structural fold is referred to as an I-fold.

Moreover, all members of the TFII-I reveal a conserved N-terminal leucine zipper (LZ) domain involved in dimerization (Fig. 2). Even if the function of the LZ in TFII-I family members is not known, a truncated mutant of TFII-I deleting the N-terminal 90 amino acids containing the LZ cannot bind DNA (Roy, 2001). This failure is not involved in dimerization of the mutant forms. Interestingly, the N90mutant does not interact with the native Δ-isoform but interacts with the β-isoform. These observations suggest that the LZ is buried in the Δ-isoform, which presumes a constitutive "closed" conformation and which alters signal-induced "opening" to form stable dimers. Conversely, the β-isoform is constitutively open and forms dimers. Because the Δ-isoform is translocated to nucleus by signal, while the β-isoform resides constitutively in the nucleus, it is predicted that dimerization is required for nuclear translocation. According to this model, while the I-fold domain initiates dimerization, LZ stabilizes such dimeric interactions regulated by extracellular signaling (Roy, 2001). A recent study certify the essential role of the N-terminal region in TFII-I. Lacking the N-terminal 140 amino acids, mutant mice exhibit serious craniofacial abnormalities (Lucena et al., 2010).

Provided that TFII-I binds to both Inr/Inr-like elements and E-box elements (Roy et al., 1997), it is also interesting to elucidate its DNA binding domains. Preceding helix 1 in repeat 2, there is a stretch of basic residues (amino acids 301–306) functioning as a DNA binding domain since lacking these residues abrogated binding to Inr/Inr-like

elements at the Vβ and c-fos promoters (Roy, 2001). It is currently not known whether the same residues also contribute to the E-box binding. TFII-I family member Ben (also called MusTRD1, GTF2IRD1, GTF3) contains 5 repeats (Fig. 2) and shows surprising binding pattern (Hinsley et al., 2004). While isolated repeats 2, 4 and 5 bound similar sequences in SELEX site selection assays, repeat 3 showed more loose sequence requirements and repeat 1 did not bind DNA at all. Individual repeats 4 and 6 of TFII-I were also subjected to site selection, even if these isolated repeats exhibited mild DNA binding activity (Vullhorst and Buonanno, 2005). It is burdensome that the stoichiometry of DNA bound TFII-I (and other family members) is currently unknown. These results suggest that different TFII-I members might use different DNA binding mechanisms and that each member might have more than one DNA binding domain. Together, It doesn`t seem that the TFII-I family proteins follow classical protein-protein and/or protein-DNA interaction patterns (Anantharaman et al., 2011).

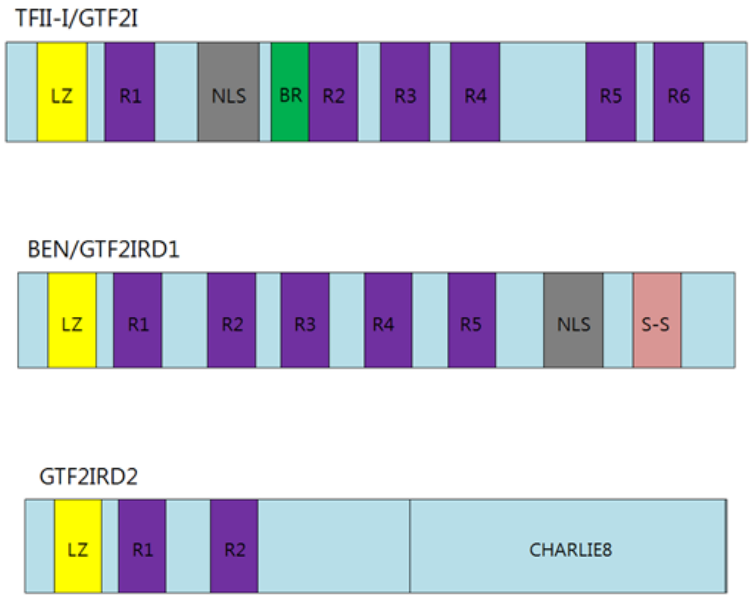


Figure 2. Schematic of the TFII-I family transcription factors (Ananda L. Roy, 2011).

TFII-I (GTF2I) has 6 repeats (R1-R6) with the NLS and BR preceding R2. BEN (GTF2IRD1/MusTRD1/GTF3) has five repeats with the NLS located toward the C-terminal region. The S-S is a serine stretch.

The third member, GTF2IRD2 has only two repeats (R1 and R2) and does not have any NLS. The N-terminal region of this member shares 75% identity with TFII-I and resembles a truncated TFII-I. The N-terminal leucine zipper (LZ) is well conserved among the three members.

2-2. Roles of TFII-I in growth factor signaling

TFII-I was shown to be necessary for mitogen-induced transcriptional activation of the c-fos gene by binding to several sites on the promoter along with SRF and STAT1/STAT3 (Grueneberg et al., 1997; Kim et al., 1998). Both growth factors (e.g., epidermal growth factor, EGF; platelet derived growth factor, PDGF) and serum phosphorylate TFII-I. The tyrosine 248 (Y248) is crucial for signal-induced transcriptional activity of TFII-I and Y248 phosphorylated TFII-I is translocated to signal-induced nucleus, suggesting that this modification plays a key role in nuclear translocation of TFII-I as well ((Cheriyath et al., 2002; Roy, 2007)). Y248 phosphorylated TFII-I also physically interacts with extracellular signal regulated kinase (ERK)-1/2 including the mitogen activated protein kinase (MAPK) family (Roy, 2007). It was assumed that ERKs are transported to the nucleus by interacting with TFII-I in response to signaling. Indeed, either lacking of the TFII-I NLS or silencing TFII-I by RNAi causes cytoplasmic heap of ERKs in response to signaling (Hakre et al., 2006). These conditions remarkably prevent c-fos transcriptional activation. Importantly, reconstitution with wild type TFII-I recovers both the ERK nuclear localization and c-fos transcriptional activation.

Though TFII-I has several alternatively spliced transcripts, roles of these isoforms were not known. While the β-isoform was constitutively found in the nucleus, the Δ-isoform was predominantly

cytoplasmic. Significantly, on signaling the β-isoform became cytoplasmic and the Δ-isoform was found in the nucleus (Hakre et al., 2006). According to this subcellular switching, the β-isoform was bound to the c-fos promoter in the basal state and the Δ-isoform was bound to the same site in response to signaling. Equally, it is important for silencing of the β-isoform to result in activation of c-fos, while silencing of Δ leads to its repression (Hakre et al., 2006). Therefore, in regard to growth factor/mitogenic signaling, the two isoforms of TFII-I set a signaling change to transcriptionally regulate c-fos.

2-3. Roles of TFII-I in regulation of β-globin

As TFII-I activates transcription of various genes in response to signaling, it represses genes as well. According to its repressive function, TFII-I interacts with various transcriptional co-repressors, such as HDAC1 and 3, LSD1 and components of the polycomb repressor complexes (PRCs) (Hakimi, 2003; Crusselle-Davis et al., 2006; Tussie-Luna et al., 2002; Wen et al., 2003). It is shown that TFII-I represses β-globin gene transcription via shifting of USF and by recruiting PRC to the β-globin locus (Crusselle-Davis et al., 2006; Anantharaman et al., 2011). The mechanism by which USF and TFII-I regulate β-globin is unknown. While interacting with each other, they exhibit opposite activities in erythroid cells. USF interacts with transcriptional co-activator p300, and TFII-I interacts with Suz12 component of the PRC2 co-repressor complex. If concentrations of

USF are low in erythroid progenitor cells, USF may form heterodimers with TFII-I as well. In the end, It contributes to the repressive activity of TFII-I.

2-4. Roles of TFII-I in regulation of angiogenesis

It was reported that TFII-I regulates vascular endothelial growth factor receptor-2 (VEGFR2) gene (Wu and Patterson, 1999). VEGFR2 (Kdr/Flk1) acts as the primary mediator of VEGF signaling in endothelial cells, and therefore regulates angiogenesis. TFII-I binds to the Inr element of the VEGFR2 gene both *in vitro* and *in vivo* and silencing of TFII-I causes decreased VEGFR2 expression (Wu and Patterson, 1999; Jackson et al., 2005). As these reports show that TFII-I activates VEGFR2 via the Inr element, a recent study shows that TFII-I represses VEGFR2 expression and angiogenesis (Mammoto et al., 2009). This study shows that p190Rho-GAP modulates capillary network formation *in vitro* in human microvascular endothelial cells and retinal angiogenesis *in vivo* by balancing activities between TFII-I and GATA2 that regulates transcription of VEGFR2. Thus, cytoplasmic accumulation of TFII-I by p190 activates VEGFR2 expression, as retention of GATA2 inhibits it.

3. Transcription factor p53

3-1. Modes of transcriptional regulation by p53

3-1-1. Activation through direct binding

Most p53-activated genes have at least one DNA-binding site which matches the consensus p53 response element. Through protein-protein interactions, p53 can bind to and recruit general transcription proteins (TATA-binding protein-associated factors (TAFs)) to the promoter-enhancer region of p53-regulated genes to induce transcription (Thut et al., 1995; Farmer et al., 1996).

3-1-2. Direct and indirect repression

In some genes, the binding of p53 to its RE directly represses that gene. At present, three methods of direct p53-mediated repression are known: first, binding-site overlap (steric hindrance); second, p53 squelching of transcriptional activators; and third, p53-mediated recruitment of histone deacetylases (HDACs).

The p53-mediated repression by steric hindrance includes sequence-specific DNA binding by p53 overlapping the binding site of another transactivating protein. Examples of genes repressed by the method of p53 steric hindrance include AFP (α-fetoprotein), BCL2 (B-cell lymphoma-2) and HBV (hepatitis B virus). In these examples, the corresponding activators that are obstructed by DNA-

Table 1. Cluster sites regulated by p53 (Riley et al., 2008).

Gene name(s)	Short description	Number of half-sites
BTG2 (TIS21)	BTG family protein-2	3
CDKN1A (p21)	Cyclin-dependent kinase inhibitor-1	2.5
DDB2	Damage-specific DNA-binding protein-2	4
GML	GPI-anchored molecule-like protein	3
HRAS (c-Ha-Ras)	Harvey rat sarcoma viral oncogene homologue	8
IGFBP3	Insulin-like growth factor binding protein-3	11
MDM2	Transformed 3T3-cell double minute 2	4
PCNA	Proliferating cell nuclear antigen	5
SH2D1A (SAP)	SH2 domain protein-1A, Duncan disease SH2 protein	4
TP53I3 (PIG3)	Tumour protein p53-inducible protein-3	7.5
TP73 (p73)	Tumour protein p73	3
TRPM2	Transient receptor potential cation channel M2	3
TYRP1 (TRP1)	Tyrosinase-related protein-1	6
VDR	Vitamin D (1,25-dihydroxyvitamin D3) receptor	3
HBV	Hepatitis B virus	3

bound p53 are FOXA1 (forkhead box A1), POU4F1 (POU domain class 4 transcription factor-1) and both RFX1 (regulatory factor X1) and ABL1 (Abelson tyrosine kinase)(Ori et al., 1998; Budhram-Mahadeo et al., 1999; Lee, et al., 1999). The p53 squelching (inactivation) of other DNA-bound and DNA-unbound activators occurs through p53-mediated protein-protein interactions. Examples of p53 squelching of other transactivating genes are cyclin B1, TERT (telomerase reverse transcriptase), IGF1R (insulin-like growth factor receptor-1), ALB (albumin) and MMP1 (matrix metallopeptidase-1). The corresponding DNA-bound proteins that are inactivated by direct p53 binding are transcription factors Sp1, CEBPβ and AP1 (activator protein-1)(Ohlsson et al., 1998; Kubicka et al., 1999; Kanaya et al., 2000; Sun et al., 2004; Innocente and Lee, 2005). Owing to the observation that p53 binds the transcription machinery proteins TBP (TATA-box-binding protein), TAF6 (TBP-associated factor-6, also known as TAFII70), TAF9 (also known as TAFII31) and others *in vitro*, it was initially believed that p53 repression was achieved through p53 binding and suppression of these TATA-box-bound basal factors *in vivo* (Seto et al., 1992; Truant et al., 1993; Thut et al., 1995; Farmer et al., 1996).

p53 binding to the repressor protein SIN3A results in the p53-mediated recruitment of HDACs (Murphy et al., 1999). After p53-mediated recruitment to the promoter-enhancer region of a gene, HDAC1 deacetylates Lys residues of histones in chromatin, thereby

repressing gene transcription57 (Harms and Chen, 2007). Examples of genes repressed by this mechanism include MAP4 (microtubule-associated protein-4), STMN1 (stathmin-1) and the heat-shock protein HSP90AB1 gene (Murphy et al., 1999; Zhang et al., 2004).

There are two accepted means of indirect p53-mediated repression. The first comes about by p53-mediated activation of CDKN1A, which in turn suppresses the cyclin D–CDK4 complex through direct binding. The consequence of this suppress of cyclin D–CDK4 is without hyperphosphorylation of the retinoblastoma (RB) protein from the G1 stage of the cell cycle (Lohr et al., 2003). Unphosphorylated RB represses the function of the E2F family of transcription factors through direct binding (forming an E2F–DP1–RB complex), thereby suppressing the many downstream targets of E2F (including cyclin E, cyclin A, DNA polymerase and thymidine kinase) and stopping the cell cycle in G1 phase.

4. Transglutaminase

The transglutaminase (TGases) are enzymes that catalyze Ca^{2+}-dependent acyl transfer reactions of glutamine side chains in proteins (Lorand and Graham, 2003). TGase-mediated modifications of glutamine residue happen in three ways (Esposito and Caputo, 2005; Figure 3): The first reaction is crosslinking reaction, which happens between the ɣ-carboxamide group of a lysine residue in a polypeptide (acyl acceptor, amine donor), and produces isopeptide

linkage between two proteins; The second reaction is polyamine incorporation, which appends polyamines to γ-carboxamide moiety of a glutamine residue; and The third reaction is deamidation, which removes amide group of glutamine residue and converts glutamine to glutamate.

Lately, nine TGase isoforms have been identified in human (Esposito and Caputo, 2005), (1) factor Ⅷ, which is expressed in blood and mediates fibrin crosslinking during blood coagulation; (2) TGase 1, TGase 3 and TGase 5, which are mostly expressed in epithelial cells and participate in formation of cornified cell envelope during keratinocyte differentiation; (3) TGase 2, which is expressed in most tissue types; (4) TGase 4, which is expressed in prostate gland and produces a post-coital vaginal plug of rodents; (5) TGase 6 and TGase 7, whose tissue distribution and function is unknown; and (6) band 4.2, which has no enzymatic activity due to absence of cysteine at active site of enzyme and participates in maintenance of erythrocyte membrane integrity. Characteristics of nine TGase are summarized in Table 2.

All TGase members have common protein structure comprising four domains and have conserved amino acid sequences at active site (G-Q-C-W-V) (Lorand and Graham, 2003). Among amino acid sequences around the active site, Cysteine, participating in the formation of thioester intermediate with substrate, is the most important residue for enzymatic activity. Comparison of the amino

acid sequences around the active site in several TGase is shown in Figure 4. Determination of three dimensional structures for TGase 2, TGase 3 and Factor Ⅷ revealed that catalytic triad cysteine, histidine and aspartate are required for transamidation reaction (Liu et al., 2002; Ahvazi et al., 2004; Pedersen et al., 1994). Therefore, human TGases are the members of papain-like superfamily of cysteine protease (Murzin et al., 1995). However, Despite common structure and sequence in substrate binding site region, the charge distribution differs among various isoforms, which may explain substrate specificities and the specialized function of each isoenzymes (Esposito and Caputo, 2005).

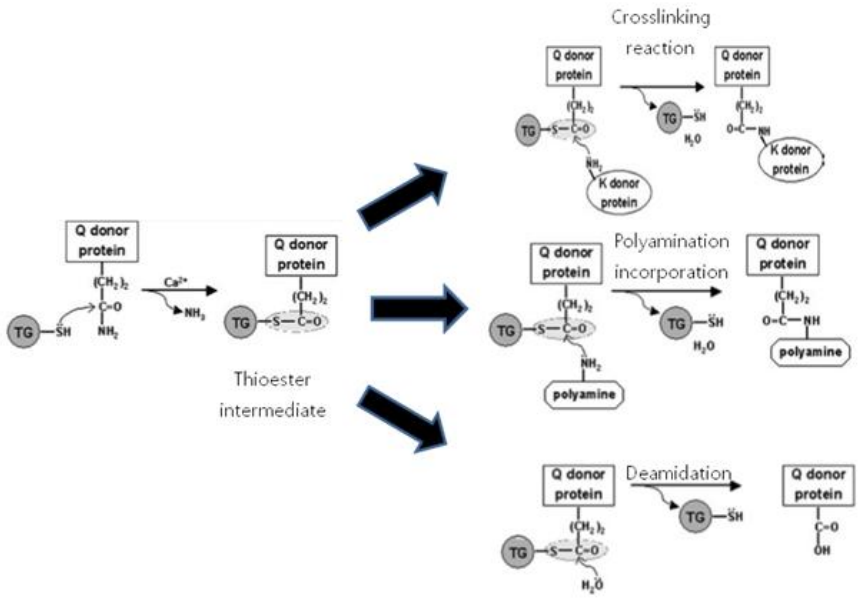


Figure 3. Three kinds of transglutaminase reaction

Transglutaminase catalyzes three kinds of reation on glutamine residue; crosslinking, polyamination and deamidation. (Esposito and Caputo 2005)

Table 2. Properties of nine transglutaminase

Protein	Residues (Mr,kDa)	Tissue expression	Localization	Function	Gene	Gene map locus
FXIII subunit A(FXIII)	732(83)	Platelets, astrocytes, dermal dendritic cells, chondrocytes, placenta, plasma, synovial fluid	Cytosolic, extracellular	Blood coagulation, bone growth	F13A1	6p24-25
TG1	814(90)	Keratinocytes, brain	Membrane, cytosolic	Cell-envelope formation	TGM1	14q11.2
TG2	686(80)	Ubiquitous	Cytosolic, nuclear, membrane, cell surface, extracellular	Multiple	TGM2	20Q11-12
TG3	692(77)	Squamous epithelium, brain	Cytosolic	Cell-envelope formation	TGM3	20q11-12
TG4	683(77)	Prostate	Unknown	Semen coagulation in rodent	TGM4	3q21-22
TG5	719(81)	Ubiquitous except for the CNS and lymphatic system	Unknown	Unknown	TGM5	15q15.2
TG6	Unknown	Unknown	Unknown	Unknown	TGM6	20q11
TG7	710(?)	ubiquitous	Unknown	Unknown	TGM7	15q15.2
Band4.2	690(72)	Red blood cell,, bone marrow, fetal liver and spleen	Membrane	Membrane skeletal component	EPB42	15q15.2

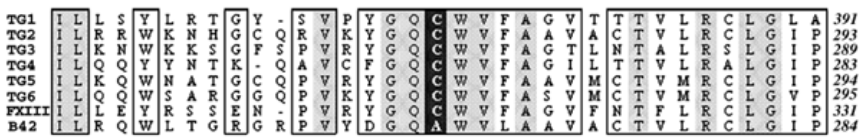


Figure 4. Comparison and contrast of amino acid sequence of human transglutaminase (black box: active site) (Esposito and Caputo, 2005)

4-1. Tissue distribution and subcellular localization

TGase 2 is the unique enzyme in that it is ubiquitously expressed (Lorand and Graham, 2003). Owing to its high expression levels in endothelium and smooth muscle cells, TGase 2 showed wide spread organ distribution in immunohistochemical study. In addition, it is abundantly expressed in cells such as mesangial cells, renomedullary interstitial cells, thymic subcapsular epithelium, and colonic pericryptal fibroblasts (Thomazy and Fesus, 1989). However, it is expressed at very low levels in some cells, such as neurons and skeletal muscle cells (Lu and Davies, 1997).

TGase 2 was firstly known as cytosolic protein, so called cytosolic TGase (TGc). However, TGase 2 has been detected in several subcellular compartments such as nucleus, plasma membrane and extracellular matrix (Fesus and Piacentini, 2002). In one cell, the majority(80%) of TGase 2 is localized in cytosol, 10-15% in plasma membrane and 5% in nuclear membrane (Lorand and Graham, 2003).

Nuclear TGase 2 can be activated by Ca^{2+}-mobilizing agent and modify different proteins from cytosolic substrates (Lesort et al., 1998). Cytosolic TGase 2 exaggerated thapsigargin-induced apoptosis, whereas inactive nuclear TGase 2 (active site cysteine is mutated) diminished apoptosis (Milakovic et al., 2004).

Although TGase 2 is found in extracellular matrix (ECM), it lacks a signal peptide for secretion. Due to absence of leader sequence, TGase 2 is not exported outside by typical endoplasmic

reticulum/Golgi-dependent pathway (Verderio et al., 2004). For externalization, TGase 2 requires an intact N-terminal fibronectin (FN) binding site (Gaudry et al., 1999). Additionally, the secretion of TGase 2 is dependent on its enzyme activity and non-proline cis peptide bond, for mutation of active site cysteine (C277S mutant) and cis peptide bond tyrosine (Y274A) attenuate its externalization (Balklava et al., 2002).

4-2. Protein structure

The detailed structure of human TGase 2 was determined by X-ray crystallography as dimerized and GDP-bound form (Liu et al., 2002; Fig. 5). Like other TGases, TGase 2 has four distinct domains: N-terminal β-sandwich domain, transamidation catalytic core domain (containing enzymatic active site), and C-terminal β-barrel 1and 2 domain (Griffin et al., 2002). In human TGase 2, each domain includes amino acid Met-1 to Phe-139, Ala-147 to Asn-460, Gly-472 to Tyr-583, and Ile-591 to Ala-687, respectively (Liu et al., 2002). Whereas β-sandwich and two β-barrel domains consist of β-structures, catalytic core domain shows mainly α-helical secondary structures (Casadio et al., 1999).

N-terminal β-sandwich domain is composed of an initial flexible loop, a short helix, a separate β-strand, five antiparallel β-strands and another short strand (Griffin et al., 2002), including two fibronectin binding sites (Met-1 to Glu-8 and Trp-88 to Thr-106

(Gaudry et al., 1999; Hang et al., 2005). The catalytic core domain consists of two β-strands which participate in GDP binding, four additional β-strands and one β-turn, and the β-barrel 2 domain has seven antiparallel β-strands (Griffin et al., 2002). In the β-barrel 2 domain, there is a binding site for phospholipase Cδ1 (PLCδ1) which is involved in the transduction of α_1 -adrenergic signals (Hwang et al., 1995). The schematic representation of each domain is shown in Fig. 5B.

According to crystallographic study, the guanine nucleotide (GTP or GDP) binding site lied in a gap between the catalytic core and β-barrel 1 domain. The major contacting domain was β-barrel 1 domain, in which, the end of the first β-strand, the loop between first and second strand and the last β-strand were involved. The catalytic core domain also included two residues (Lys-173 and Phe-174) contacting with guanine base. Val-479 and Arg-580 formed hydrogen bond with phosphates of GDP, and Ser-482 and Tyr-583 were participates in hydrogen bond with guanine base of GDP. The GDP-bound form of TGase 2 showed compact and inactive state, because two loops of the β-barrel 1 domain blocked substrate access to the catalytic triad and Tyr-516 made a hydrogen bond with active site Cys-277(Liu et al., 2002).

From the comparison of TGase 2 TGase 3 structure, TGase 2 was suggested to have three putative Ca^{2+} binding sites: site 1 (Gly226, Asn229, Asn231, and Asp233), site 2 (Asp306, Asn308, Asn310,

Leu312, and Glu329) and site 3 (Asn398, Ser419, Glu447, and Glu452). These binding sites were all needed for full activation of TGase 2 and mutation of site 2 most profoundly attenuated the enzymatic activity (Datta et al., 2006). The Ca^{2+}-bound TGase 2 was suggested to undergo conformation change for enzymatic activation substrate access to active site (Griffin et al., 2002).

4-3. Regulation of TGase 2 transamidation activity

Two main factors which transamidation activity of TGase 2 are Ca^{2+} and guanosine nucleotides (GTP and GDP). The effect of these two factors on TGase 2 activity is just the opposite: Ca^{2+}activates enzymatic activity (Ka~1mM; Piper et al., 2002), but GTP and GDP inhibit (Kdaapp; Datta et al., 2006; Lai et al.,1998). When not stimulated, TGase 2 is enzymatically dormant in cell cytosol, where the GTP concentration is about 100uM and the free calcium concentration is approximately 100uM (Siegel and Khosla, 2007). Depletion of GTP by GTP-lowering drug tiazofurin and elevation of intracellular Ca^{2+} by Ca^{2+}-mobilizing drug maitotoxin increased intracellular TGase activity (Zhang et al., 1998).

As mentioned above, GTP-bound TGase 2 is compact and stable. This conformation inhibits the access of substrates to active site and makes the enzyme catalytically inactive. This allosteric inhibition was achieved via masking a conformationally destabilizing switch

residue, Arg-579 and forming unusual hydrogen bond between Tyr-516 and active site Cys-277 (Begg et al., 2006).

Ca^{2+}-bound TGase 2 was proposed as active conformation similar to covalent inhibitor-bound structure. In crystallographic study, inhibitor-bound TGase 2 showed open conformation by extension of two β-barrel domains from core domain, which made the enzyme a rod-like shape. This conformation formed hydrophobic tunnel to active site cysteine bridged by two tryptophans (Trp-241 and Trp-332) and enabled substrates to access the active site (Pinkas et al., 2007).

Another potential regulatory factor of TGase 2 activity is phospholipid. Purified guinea pig liver TGase 2 interacted with phospholipid vesicles (Fesus et al., 1983). Sphingosylphosphocholine (lyso-SM), a minor membrane phospholipid constituent, activated TGase 2 activity at relatively low Ca^{2+} level and reversed the inhibitory effect of GTP (Lai et al., 2001). TGase 2 could be nitrosylated on several cysteine residues including active site cysteine, and nitrosylation of TGase 2 resulted in inhibition of enzyme activity. This inhibitory effect increased on Ca^{2+}-dependent manner, which implies that the Ca^{2+} concentration affects TGase 2 nitrosylation states. In addition, nitrosylation of TGase 2 also enhanced the inhibitory effect of GTP and inhibited ADP-induced platelet aggregation.

The pH can affect TGase 2 enzymatic activity determination. At above pH7, TGase 2 kinetically favored the transamidation reation over deamidation, but lowering of pH increased the rate of the deamidation reaction by TGase 2 (Fleckenstein et al., 2002).

4-4. Functions of TGase 2

Since TGase2 can bind guanine nucleotides and hydrolyze GTP (GTPase activity), it has been considered as G-protein in signal transduction pathway. TGase 2 was also called as Ghα (Nakaoka et al., 1994) and formed heterodimeric complex with Ghβ, calreticulin (Feng et al., 1999). TGase 2 interacted with G-protein coupled receptors (GPCRs) such as α1-adrenergic receptors (α1-AR), oxytocin receptors and thromboxane A2 receptors (Mhaouty-Kodja, 2004). The two domains of TGase 2, Arg-564 to Asp581 and Gln-633 to Glu-646 seem to be important for receptor binding. The ligand binding to these receptors resulted in activation of TGase 2 as G-protein by exchange of GDP to GTP and dissociation from Ghβ.

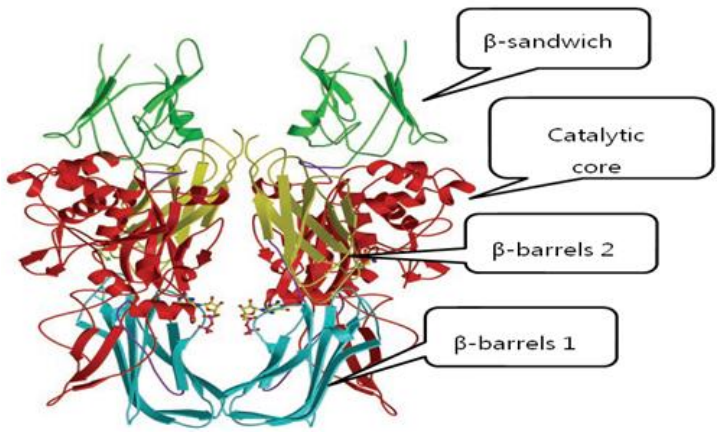
On the cell surface, TGase 2 interacts with β1 and β3 integrins and acts integrin-associated coreceptor for fibronectin binding (Akimov et al., 2000) TGase 2 enhanced phosphorylation of focal adhesion kinase (FAK) induced by fibronectin-integrin binding and mediated cell adhesion and spreading. This effect of TGase 2 was independent of its transamidation activity. In addition, TGase 2 induced clustering of β1 integrin on the cell surface and increased

integrin-induced RhoA and ROCK (Rho-associated coiled-coil containing serine/threonine protein kinase) activation, which is associated with cell polarization and stress fibers formation (Janiak et al., 2006).

Purified TGase 2 was reported to have protein disulphide isomerase (PDI) activity (Hasegawa et al., 2003). TGase 2 reactivated completely reduced RNase A and this effect was suppressed by bacitracin, the conventional PDI inhibitor. The PDI activity of TGase 2 did not require transamidation active site cysteine and Ca^{2+}. In addition, this enzymatic activity was not inhibited by GTP. TGase 2 was suggested to act as PDI in the mitochondrial respiratory chain complexes and regulate ATP production in mitochondria (Mastroberardino et al., 2006).

TGase 2 was also proposed to have intrinsic serine/threonine kinase activity. It was firstly identified as kinase responsible for insulin-like growth factor-binding protein-3 (IGFBP-3) phosphorylation (Mishra and Murphy, 2004), and p53, histone proteins and retinoblastoma protein (Rb) were also reported to be phosphorylated by TGase 2 (Mishra et al., 2007; Mishra and Murphy, 2006; Mishra et al., 2006). This kinase activity of TGase was inhibited by Ca^{2+} and TGase 2 transamidation activity inhibitor such as cystamine and monodansyl cadaverine (Mishra and Murphy, 2004), and augmented via phosphorylation of TGase 2 by protein kinase A (Mishra et al., 2007).

(A)



(B)

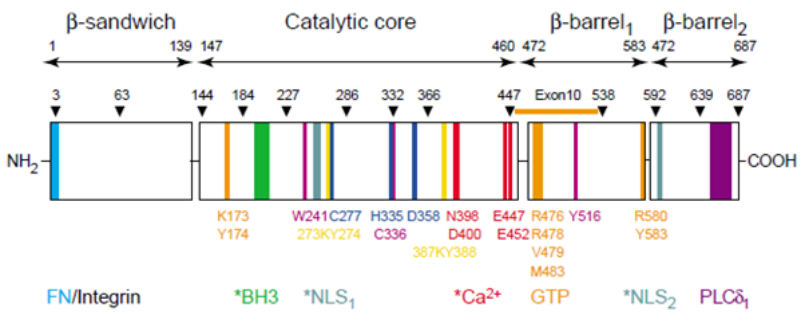


Figure 5. Structure of transglutaminase 2

(A) Transglutaminase 2 is composed to four domain; β-sandwich, Catalytic core, β-barrels 1 and β-barrels 2 (Liu, Cerione et al. 2002)

(B) Schematic representation of domain structure of transglutaminase 2 (Fesus and Piacentini 2002)

4-5. Physiological function

It has been proposed that TGase 2 is involved in apoptosis by inducing irreversible cross-linking of cellular proteins and restraining the leakage of intracellular materials from apoptotic cells (Fesus et al., 1991). TGase 2-overexpressed neuronal cells were more sensitive to apoptosis due to TGase 2-induced changes of mitochondria function (Piacentini et al., 2002). However, there was no difference in apoptosis of TGase 2 null cells compared to wild type cells (De Laurenzi and Melino, 2001). Moreover, increase of TGase 2 suppresses apoptosis by doxorubicin treatment and serum deprivation (Antonyak et al., 2004; Herman et al., 2006). Therefore, it was suggested that TGase 2 have pro- and anti-apoptotic functions via the type of cells, apoptotic stimuli and executed enzymatic activities (Fesus and Szondy, 2005; Kotsakis and Griffin, 2007). Transamidation activity of TGase 2 showed both promotion and inhibition of apoptosis, but GTP-bound form and cell surface TGase 2 usually diminished cell death.

Since TGase 2 is exported to outside of cells, and extracellular space is favorable for activation of TGase 2, it has been suggested to be involved in ECM deposition and stabilization by crosslinking extracellular proteins (Ientile et al., 2007). ECM-associated proteins such as collagen, fibronectin, fibrinogen, vitronectin, osteopontin, nidogen, laminin, osteonectin and osteocalcin were modified by TGase 2 and this modification confer resistance for mechanical and

proteolytic degradation on ECM (Griffin et al., 2002). TGase 2 was related to activation of TGF-β, which is involved in ECM deposition and cell differentiation by mediating covalent linkage between the latent TGF-β binding protein 1 (LTBP-1) and ECM (Nunes et al., 1997). In addition, cell surface TGase 2 was associated with integrin for fibronectin binding and mediated cell adhesion and migration independently of its crosslinking activity (Akimov et al., 2000). As an integrin-associated coreceptor, TGase 2 facilitated fibronectin assembly mediated by α5β1 integrin (Akimov and Belkin, 2001). These evidences suggest that TGase 2 is associated with ECM regulation and wound healing process.

Inside of the cells, TGase 2 has been suggested to be involved in cytoskeletal regulation. Microfilaments including actin, myosin, spectrin, thymosin and troponin T and intermediate filaments such as keratin, vimentin and neurofilaments can be modified by TGase 2 (Fesus and Piacentini, 2002). In addition, TGase 2 interacted with β-tubulin, one of microtubular proteins (Piredda et al., 1999) and crosslinked microtubule-binding protein tau (Murthy et al., 1998). TGase 2 also can activate RhoA and ROCK by direct polyamination of RhoA or signal transduction from integrin-associated TGase 2 and these activations result in phosphorylation of vimentin and the formation of stress fiber (Singh et al., 2001; Janiak et al., 2006) .

Furthermore, TGase 2 was involved in signal transduction from GPCRs including α1-adrenergic receptors (α1-AR), oxytocin

receptors and thromboxane A2 receptors (Mhaouty-Kodja, 2004), chromatin modification by crosslinking several core histones (Kim et al., 2001) and matrix mineralization by affecting chondrocyte and osteoblast differentiation (Nurminskaya and Kaartinen, 2006).

Purpose

The ribosomal proteins are major constituents of ribosomes catalyzing protein synthesis in the cytoplasm. Ribosomal protein L7a (RPL7a) consist of a globular surface-exposed RNA-binding domain binding to the rRNA core to stabilize its structure. In addition, ribosomal proteins have a special function outside the ribosome involving various cellular processes such as replication, transcription, RNA processing, DNA repair and even inflammation.

Abrupt activation of TGase 2 is associated with various disease conditions such as cataract, fibrosis, neurodegeneration and cancer. Precise regulation of TGase 2 activity is important for cellular homeostasis. Previous study showed that RPL7a is a negative regulator of TGase 2 activity. Therefore, the regulation of RPL7a expression is important in TGase 2 activity and TGase 2-associated disease process.

The aim of this study is to identify transcription factors that regulate the expression of RPL7a.

Material and Method

1. Cell culture

Human lung cancer cell A549, breast cancer cells MCF7 were maintained with 10ml of RPMI (Rosewell Park Memorial Institute) medium and Hela, colon cancer cell HCT116 (p53+/+) maintained with 10ml of Dulbecco's Modified Eagle's Medium (DMEM) in culture in 100pi dishes supplemented with 10% fetal bovine serum (FBS) (HyClone) and 1% Penicillin-Streptomycin (GIBCO). They were grown at 37 C° in humidified incubator containing 5% CO_2

2. Construction of the RPL7a (-1147 to +29) promoter construct

Primers were designed for amplification of the region from −1147 to +29 (with the transcription start site designated +1) of the RPL7a gene.

Primer sequences were:

RPL7a F: 5'- GG GGTACCCTCCCATCCGAAGAGTCC-3 (KpnI site is underlined) and

RPL7a R: 5'-CCGCTCGAGTTGGGCGGCGGG-3' (XhoI site is underlined)

Promoter PCR products were excised with KpnI and XhoI restriction enzymes for subcloning in to the luciferase reporter

plasmid pGL3-Basic(Promega). Cloned promoter constructs were verified by DNA sequencing

3. Generation of RPL7a promoter deletion constructs

Promoter deletion constructs were generated using either restriction sites common to both the cloned promoter fragment and the pGL3-Basic multiple cloning site.

Primer sequences were:

5'-GGGGTACCCTCCCATCCGAAGAGTCC-3' (KpnI for the -1147 to + 29 construct),

5'-GGGGTACCCGTGATGGCAGGCGCCTG-3' (KpnI for the -838 to + 29 construct),

5'-GGGGTACCATGGTTCCCCGGTGCGCC-3' (KpnI for the -647 to + 29 construct),

5'-GGGGTACCGCGGACTCCTGGACGCAC-3' (KpnI for the -465 to + 29 construct),

5'-GGGGTACCTCGGCCTAGGGCGGGGTG-3' (KpnI for the -252 to + 29 construct),

5'-GGGGTACCATAGGAATGCTGTTGCTT-3' (KpnI for the -90 to + 29 construct; KpnI restriction site is underlined) and

the RPL7a R primer.

5'-CCGCTCGAGTTGGGCGGCGGG-3' (Xho1 restriction site is underlined)

4. site-directed mutagenesis

Mutations in potential transcription factor binding sites in the RPL7a (-90to+29) promoter construct were prepared by site-directed mutagenesis, using mutagenic primers. Primer sequences were:

1.c-ETS-1 mut: 5'- cctcgatttttagctttatagg**gc**tgctgttgctttaaatccg-3'and

5'- cggatttaaagcaacagca**gc**cctataaagctaaaaatcgagg-3'

2. TFII-I(2) mut: 5'-ggaatgctgttgctt**ccccc**ccgaaatcccgtgcc-3'and

5'-ggcacgggatttc**ggggg**ggaagcaacagcattcc-3'

3. c-Myb mut: 5'-cccgtgccggtatc**tct**tctcgcgatctccgag-3' and

5'-ctcggagatcgcg**aga**agagataccggcacggg-3'

4. TFII-I(4) mut: 5'-ggtatcaactctcgc**cggt**tccgaggccgcatac-3' and

5'- gtatgcggcctcgga**accg**gcgagagttgatacc-3'

5. E2F-1 mut: 5'-cgcgatctccgaggc**at**catacatattaccc-3' and

5'-gggtaatatgtatg**at**gcctcggagatcgcg-3'

6. TATA box mut: 5'-gaggccgcatacata**ggg**cccacaattccctttc-3' and

5'-gaaagggaattgtggg**ccc**tatgtatgcggcctc-3'

7. p53 mut: 5'-ctttctctctcctccatc**atct**caactcgagatctgc-3' and

5'- gcagatctcgagttgag**atgat**ggaggagagagaaag-3'

8. TFII-I(1)site mut:

5'-tctctatcgataggtaccatagga**gaa**ctgttgctttaaatccgaaatccc-3'and

5'-gggatttcggatttaaagcaacag**ttc**tcctatggtacctatcgatagaga-3'

9. TFII-I(3)site mut:

5'-ccataggaatgctgttgctttaaatccgaaa**gaa**cgtgccggtatcaact-3'and

5'-agttgataccggcacg**ttc**tttcggatttaaagcaacagcattcctatgg-3'

10. TFII-I(5)site mut:

5'-ccgaggccgcatacatattacccaca**ggg**ccctttcctttctctc-3'and

5'-gagagaaaggaaagggc**ccc**tgtgggtaatatgtatgcggcctcgg-3'

Site-directed mutagensis of RPL7a promoter (-90 to +29 construct) was performed using RPL7a promoter deletion (-90 to +29 construct) construct (50ng) as a template, 10uM fresh dNTPs, 2.5 units pfu-ultra polymerase (stratagene) and 1pmol each of the following primers combination in a volume of 50ul according to manufacture`s protocol (Stratgene). Mutated RPL7a promoter (-90to+29 construct) sequences were confirmed by DNA sequencing.

5. siRNA transfection

For the effect of siRNA on RPL7a promoter activity, A549 cells of a 24-well plate were transfected with 20pM control GFP siRNA (sc-45924, Santa Cruz Biotechnology), TFII-I siRNA (sc-36643, Santa Cruz Biotechnology), and 24 h later transfected with 800ng RPL7a (-90 to +29) promoter construct and 20ng pRL-TK plasmid. Luciferase activity was measured 24 h later and expressed as relative folds to Renilla luciferase in the same extract.

6. Transient transfection and Luciferase assay

800 ng of each promoter construct was transfected into all kinds of cells of a 24-well plate, using 1.64ul Lipofectamine™ 2000

Transfection Reagent (invitrogen). To normalize for transfection efficiency the cells were co-transfected with 10 ng of the pRL-TK plasmid that encodes Renilla luciferase. Total cell lysates were prepared from cells 24 h post-transfection using Passive Lysis Buffer (Promega) and firefly luciferase activity was assayed using the Dual Luciferase Kit (Promega). Luminescence was monitored using the Glomax 96 microplate luminometer (Promega).

7. Chromatin immunoprecipitation (chIP) assay

Cells were grown to approximately 90 % confluence and protein-DNA complexes cross-linked with 1% formaldehyde for 10 min., followed by the addition of 0.125 M Glycine, pH 2.5. Cells were harvested, washed by 3 times with 10ml cold 1X PBS.

Cell pellet was suspended in 500ul of buffer A (5mM PIPES, pH8.0), KCl (85mM), NP-40 0.5%), incubated on ice for 10min and centrifuged at 5000rpm for 5min at 4 ° C to pellet the nuclei. The nuclei were resuspended in 150ul of buffer B (1% SDS, 10mM EDTA, 100mM Tris-Cl, pH8.1) on ice for 10min and sonicated to lengths of between 600 and 1000 bp. Cell lysates were centrifuged at 15000 rpm for 10min at 4 ° C, and diluted in IP buffer (1.1% Triton X-100, 1.2mM EDTA, 167mM NaCl, 16.7mM Tris-Cl, pH 8.1, 0.01% SDS, 1× Complete Protease Inhibitor, Roche). Protein-A agarose beads (repligen) were precleared in 11 mg/ml salmon sperm DNA (sigma-aldrich) at 4 ° C overnight. Chromatin was incubated with 1 μg

antibody (p53; sc-6243, Santa Cruz Biotechnology), control IgG (sc-2027, Santa Cruz Biotechnology), TFII-I (cell signalling).

Next day, immune complexes bound by the beads were incubated at 4 ° C for 3 hours and were recovered by centrifugation and washed 4 times sequentially in TSE 150 (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20mM Tris-Cl, pH 8.1, 150 mM NaCl), TSE 500 (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-Cl, pH 8.1, 500 mM NaCl), Buffer C (0.25 M LiCl, 1% NP-40, 1% Sodium Deoxycholate, 1 mM EDTA, 10mM Tris-iCl,pH 8.1) and TE (pH8.0). Bound material was eluted using elution buffer (1% SDS, 0.1 M NaHCO3) at room temperature for 20 min, and the input and eluted samples were heated at 65 ° C overnight to reverse the formaldehyde cross-links. DNA was purified and used for real-time PCR, conventional PCR using primers designed to span the respective transcription factor binding sites.

RPL7a promoter

F: 5' -GATCTCCGAGGCCGCATACATATT-3' ,

R :5' -ATAGTGCCACGGAACTACAGCTCA-3' ;

Puma

F: 5' -GCGAGACTGTGGCCTTGTGT-3' ,

R :5' -CGTTCCAGGGTCCACAAAGT-3'

RPL7a-Coding region

F: 5' -AGGAGTTAACACCGTCACCACCTT-3' ,

R :5' -TTAGCAGTCAACAGGCAAACGCAC-3'

c-fos

F : 5’ - GCAGCCCGCGAGCAGTT -3’

R : 5’ - GCCTTGGCGCGTGTCCTAATC -3’

These primers amplified a 99bp, 75bp, 106bp, 201bp product, respectively.

8. Real time-PCR

RPL7a transcript expression analysis was performed by reverse transcriptase PCR on total RNA extracted by A549 cell using TRIzol reagent (Invitrogen). RNAs were quantified by Nanodrop spectrophotometer and treated with DNase I (Ambion) following the manufacturer’s instructions. RNA samples were reverse transcribed using a complementary DNA (cDNA) synthesis kit (iNtRon biotechnology) according to the supplier’s instructions and then amplified.

RPL7a primer

F: 5’ -GCCGCCCAAGATGCCGAAAG-3’

R: 5’ -CCGGGCCAACAGTCTCTGCT-3’

β_2 -macroglobulin primer

F: 5’ -TGAGTATGCCTGCCGTGTGAAC-3’

R: 5’ -TGCTGCTTACATGTCTCGATCCC-3’

9. Western blot

The cells were lysed in a buffer containing 50mM Tris-Cl (pH8.0), 150mM NaCl, 1% triton X-100 and protease inhibitor cockail and centrifuged at 12000 x g for 10min at 4° C. The protein concentration of the supernatant was determined using the BCA method. Each sample was resolved by SDS-PAGE and transferred onto nitrocellulose membranes. After treatment for 1h with 5% skim milk in Tris-buffered saline, the membranes were incubated separately with antibodies against RPL7a, TFII-I (Santacruz), P53 and actin (sigma) for 2 h. The membranes were subsequently washed, incubated with HRP-conjugated secondary antibody and developed using a chemiluminescence substrate solution, as instructed by the manufacturer (Pierce).

10. Etoposide treatment

Etoposide (Sigma Cells) were used with 50mM for all experiments. For the analysis of the effect of etoposide on p53 protein, cells were treated with etoposide for 24 h. For analyzing the effects of etoposide on the RPL7a promoter, cells were transfected with the promoter constructs and 5 h later treated with etoposide for 24 h. For ChIP analysis of p53 binding to the RPL7a promoter, cells were treated with 50mM etoposide for 24 h. For p53 knock-down analysis, cells were transfected with control GFP siRNA-A (Santa Cruz

Biotechnology) or p53 siRNA (Santa Cruz Biotechnology) 24 h prior to etoposide treatment.

Result

1. Screening of putative binding sites for transcription factors in RPL7a promoter

Firstly, we investigated RPL7a protein expression in A549, MCF7, Hela cell lines. Western blot analysis revealed enhanced RPL7a expression in A549 and MCF7 in comparison with Hela (Fig. 6A).

To determine transcriptional regulatory mechanisms that differ from RPL7a expression in A549, MCF7, and Hela cell lines, an about 1.2 Kb region upstream of the transcription start site of the RPL7a gene was cloned into the pGL3-Basic vector for promoter analysis. The -1147 to +29 RPL7a construct showed significantly higher activity in A549 and MCF7 in comparison with Hela (Fig. 6B).

To investigate the region responsible for the differential expression of RPL7a in A549, MCF7 and Hela, a series of deletion constructs of the RPL7a promoter were generated and assayed for activity in A549, MCF7 and Hela. There was no deletion constructs showing a marked difference of activity in all cell lines. However, results showed that -90 to +29 construct displayed activity to some extent (Fig. 6C). This suggests that functional elements, necessary for RPL7a promoter activity in A549, MCF7 and Hela are likely to reside in the -90 to +29 region of the RPL7a promoter.

Since the -90 to +29 region of the RPL7a promoter was identified as important for RPL7a promoter activity in A549, MCF7 and Hela, a

bioinformatic analysis of this region was performed using TFSEARCH, PROMO software program to identify putative transcription factor binding sites. The -90 to +29 region of the promoter was identified to contain many transcription factor binding sites, of which the STAT-4/c-ETS, FoxA1/TFII-I (2), GATA-1/2(-52), c-Myb, TFII-I(4), E2F-1, TATA, p53 were investigated (Fig. 7).

To determine the function of the putative STAT-4/c-ETS, TFII-I(2), GATA-1/2(-52), c-Myb, TFII-I(4), E2F-1, TATA, p53 binding sites, these sites were mutated in the -90 to +29 promoter construct through site-directed mutagenesis and the mutated constructs transfected into A549. Mutation of TFII-I(2),TFII-I(4) sites markedly reduced RPL7a promoter activity but that of p53 increased it (Fig. 8), suggesting that TFII-I(2),TFII-I(4) and p53 have an impact on RPL7a promoter activity, and are functional elements in RPL7a promoter.

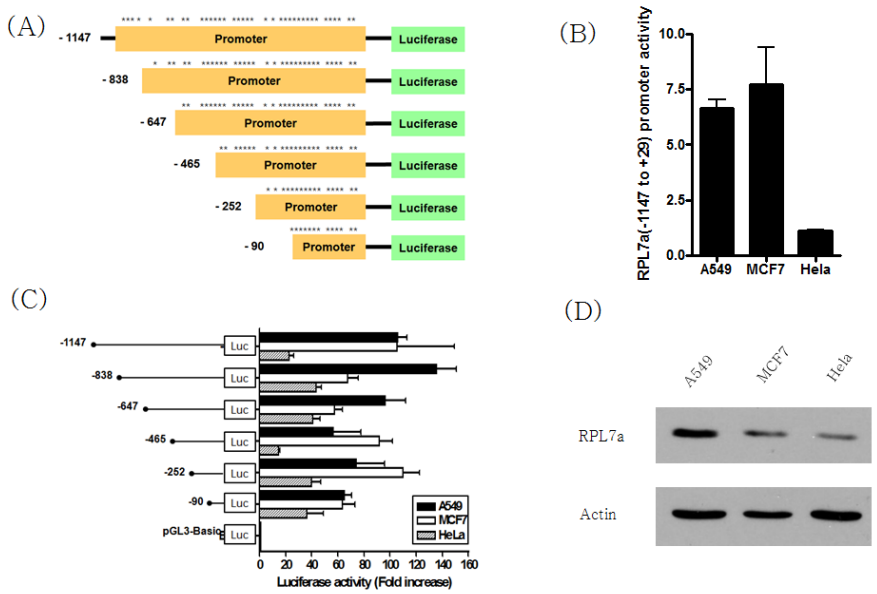


Figure 6. Identification of active promoter region of RPL7a in various cancer cell lines

(A) Schematic presentation for deletion constructs of RPL7a promoter

(B) Basal promoter activity of RPL7a promoter (−1147 to +27) determined by luciferase assay

(C) Promoter activity of deletion constructs of RPL7a promoter determined by luciferase assay.

(D) The amount of RPL7a protein is detected by western blot as described in Material and Methods. The amount of RPL7a protein varies from cell to cell.

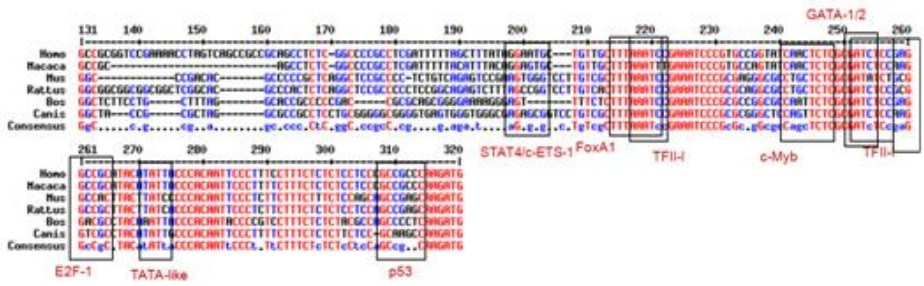


Figure 7. Putative transcription factor binding site within the −90 to + 29 region

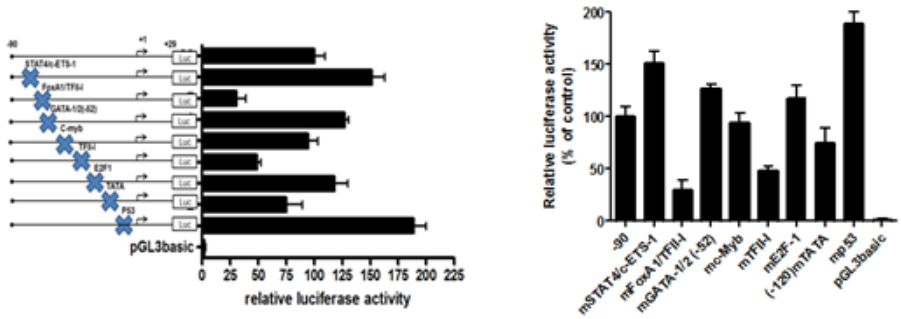


Figure 8. Identification of TFII-I and p53 as putative transcription factors within the -90 to +29 region

Transcription factor putative binding sites of RPL7a (-90 to +29) construct were modified through site-directed mutagenesis. A549 cells were transiently transfected with RPL7a (-90 to +29) construct and lucifease activity were measured at 24h post-transfection. Each experiment was done in triplicate and luciferase activity assay was described in Material and Method.

2. Repression of RPL7a transcription by p53.

As the p53 were identified as functional element within the RPL7a promoter, we next investigated whether p53 played a role in RPL7a promoter regulation. To overexpress p53, pCMV-p53 was transfected into A549 in a dose-dependent manner and (-1147 to +29) full length, (-90 to +29), (-90 to +29) p53 mutant construct was also transfected into A549. Overexpression of p53 decreased RPL7a promoter activity on (-1147 to +29) full length, (-90 to +29) constructs in a dose-dependent manner but increased it on (-90 to +29) p53 mutant construct in a dose-dependent manner (Fig. 9), confirming a role for p53 in the regulation of RPL7a promoter. Consistent with this result, RPL7a mRNA was measured in p53-overexpressed A549 to investigate effect on RPL7a transcription. When p53, compared to control vector (pCMV tag 2B), was overexpressed, RPL7a mRNA level, albeit slightly, was decreased (Fig. 10).

Next, to investigate RPL7a expression level, protein was measured in p53-overexpressed A549 by western blot. When p53, compared to control vector (pCMV tag 2B), was overexpressed, RPL7a expression level, albeit slightly, was decreased (Fig. 11).

To confirm the involvement of p53 with chemical treatment, HCT116 (p53+/+), HCT116 (p53-/-) were treated with etoposide (a potent DNA-damaging agent and known inducer of p53 activity) in a time-dependent manner. From 3h post-etoposide treatment, p53

increased in HCT116 (p53+/+). At 24h, p53 expression level is peaked. RPL7a expression level is decreased. But no change in RPL7a expression level was observed in HCT116 (p53-/-) (Fig 12).

In vivo binding of p53 to putative site was next investigated. Chromatin immunoprecipitation (chIP) assays were performed using chromatin prepared from etoposide-treated A549 and after immunoprecipitation with a p53 antibody, PCR amplication was achieved using primers designed to span putative p53 binding site. A positive control band was detected in PUMA promoter. It was likely that p53 did directly bind to RPL7a promoter (Fig. 13).

These findings suggest that as directly binding to RPL7a promoter, p53 plays a role in repressing RPL7a transcription.

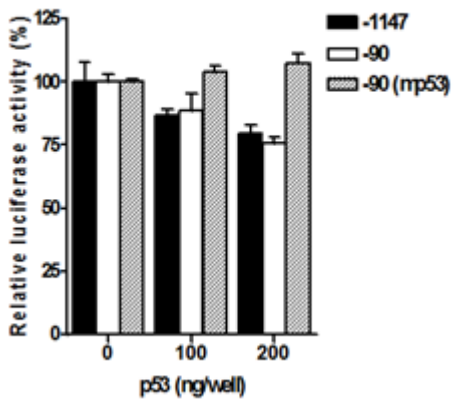


Figure 9. Overexpression of p53 decreases RPL7a promoter activity

A549 cells were transiently co-transfected with p53 and RPL7a promoter constructs (RPL7a (-1147 to +29) full length, RPL7a (-90 to +29), or mutant RPL7a (-90 to +29) defective for p53 binding (-90(mp53)). Luciferase activity was measured at 24h post-transfection. When p53 was overexpressed in a dose-dependent manner, RPL7a promoter activity decreased.

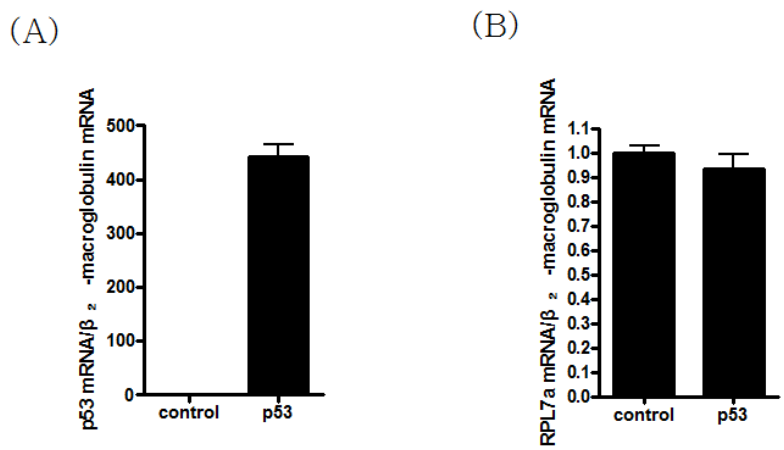


Figure 10. Overexpression of p53 decreased RPL7a mRNA level

p53 was transiently transfected into A549 cell and then after 24h, mRNA is extracted and RPL7a mRNA was measured using real time-PCR. Real time-PCR was described in Material and Method. (A) p53 mRNA was increased after overexpression of p53. (B) Overexpressed-p53 decreased RPL7a mRNA level.

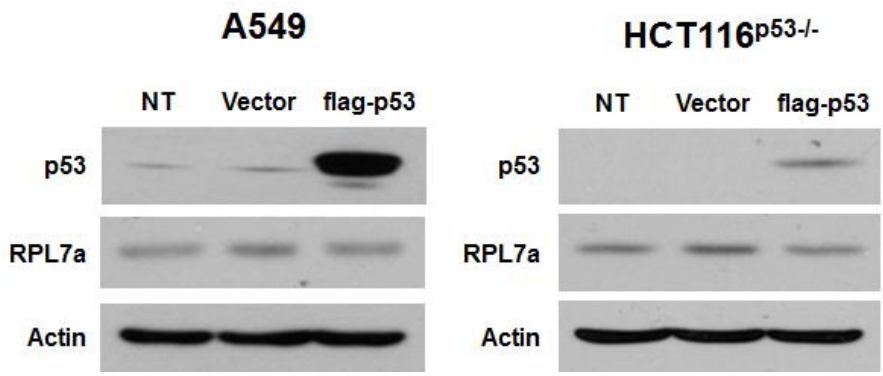


Figure 11. Overexpression of p53 decreased RPL7a protein level

FLAG-p53 was transfected into A549 cell. Cells were harvested after 24h post-transfection. RPL7a protein amount is detected by western blot as described in Material and Method. Overexpressed-p53 decreased RPL7a protein level in A549 and HCT116 (p53-/-) cells.

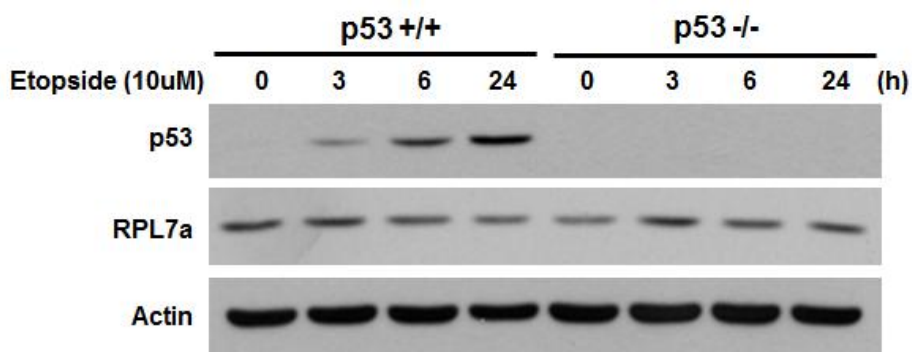


Figure 12. RPL7a protein level was decreased by etoposide treatment in p53-dependent manner

Etoposide was treated in a time-dependent manner on HCT116 (p53+/+), HCT116 (p53-/-). p53 protein level increased by etoposide treatment, but RPL7a protein level decreased only in p53 wild-type cells (HCT116 (p53 +/+)).

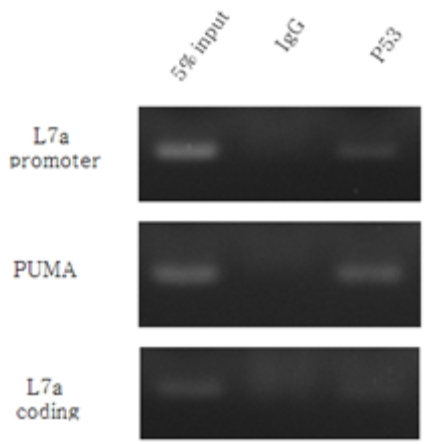


Figure 13. p53 directly binds to the RPL7a promoter region in A549 cells.

p53 binding over promoter region of RPL7a was deduced from chIP as described in Material and Method. Positive control was selected by PUMA primers. Negative control was selected by RPL7a coding region primer. Each eluted DNA was amplified by conventional PCR. p53 directly bound RPL7a promoter region.

3. Augmentation of RPL7a transcription by TFII-I

To investigate the role of TFII-I as a candidate of transcription factor affecting RPL7a promoter activity, Each TFII-I binding site, both sites were mutated in -90 to +29 promoter by site-directed mutagenesis. Both sites-mutated construct decreased RPL7a promoter activity much more than each one (Fig. 14).

As TFII-I was functional transcription factor within RPL7a promoter, we next investigated whether TFII-I played a role in RPL7a promoter regulation. Overexpression system was employed. TFII-I was transfected in a dose-dependent manner and (-1147 to +29) full length, (-90 to +29), (-90 to +29) double mutant construct also were co-transfected into A549. When TFII-I dose was 200ng, RPL7a promoter activity increased in all constructs (Fig. 15). This implies that other binding sites exist. As a result of finding other binding sites, TFII-I (1), (3), (5) binding sites were found in -90 to +29 promoter. Each TFII-I binding site was mutated. The newly found TFII-I (5) binding site decreased RPL7a promoter activity (Fig. 16) suggesting that TFII-I (2), (4), (5) binding sites play an important role in regulating RPL7a promoter.

Next, siRNAs were employed to silence TFII-I expression and effects on the RPL7a promoter activity measured. Knock-down of TFII-I resulted in a decrease in RPL7a promoter activity (Fig. 17) and then decrease of RPL7a mRNA, confirming a role for this protein in the regulation of RPL7a expression (Fig. 18).

In vivo binding of TFII-I to their respective sites was investigated. Chromatin immunoprecipitation (chIP) assays were performed using chromatin prepared from A549 and after immunoprecipitation with TFII-I antibody, PCR amplication was performed using primers designed to span putative TFII-I binding sites. A positive control band was detected in c-fos promoter. It was likely that TFII-I did directly bind to RPL7a promoter (Fig. 19). These findings indicate that TFII-I plays a role in increasing RPL7a transcription by binding directly to RPL7a promoter.

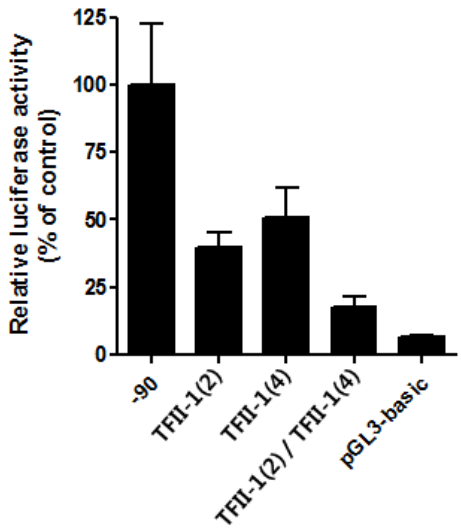


Figure 14. Mutations in two TFII-I binding sites remarkably decreased RPL7a promoter activity

A549 cells were transiently transfected with RPL7a (-90) mutant constructs. Luciferase activity was measured at 24h post-transfection. The activity of RPL7a promoter containing mutations in two TFII-I binding sites (TFII-I(2)/TFII-I(4)) decreased compared with those containing one mutation (TFII-I(2) and TFII-I(4)).

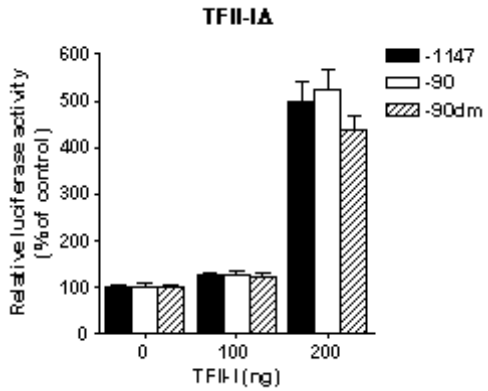


Figure 15. Overexpression of TFII-I increased RPL7a promoter activity on -90 to +29 promoter construct.

TFII-I(δ) isoform was transiently transfected into A549 cells in a dose-dependent manner. RPL7a (-1147 to +29) full length, RPL7a (-90 to +29), RPL7a (-90 to +29) double mutant construct also were transiently transfected into A549 cells. Luciferase activity was measured at 24h post-transfection. RPL7a promoter activity increased in all RPL7a promoter constructs.

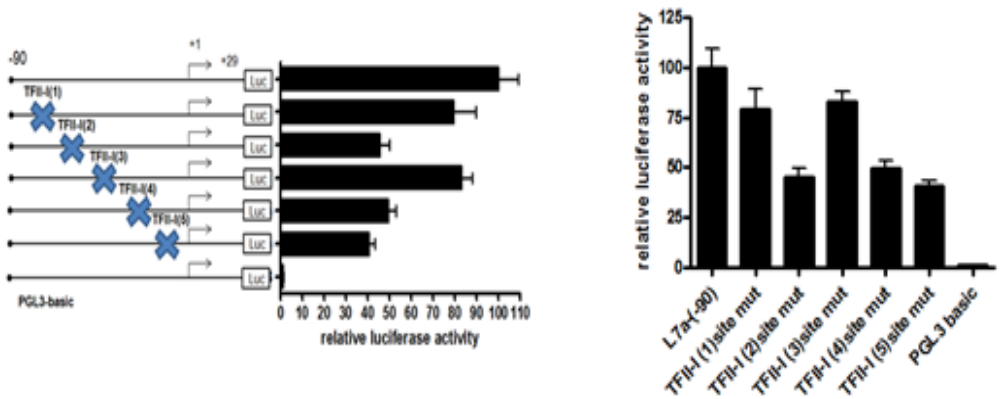


Figure 16. Three putative TFII-I binding sites are involved in RPL7a promoter activity.

There are five putative TFII-I binding sites in RPL7a promoter, and all of them were modified by site-directed mutagenesis (TFII-I (1) to (5)). Mutants containing TFII-I (2),(4),(5) binding site decreased RPL7a promoter activity. Each mutant was transiently transfected into A549 cells. Luciferase activity was measured at 24h post-transfection.

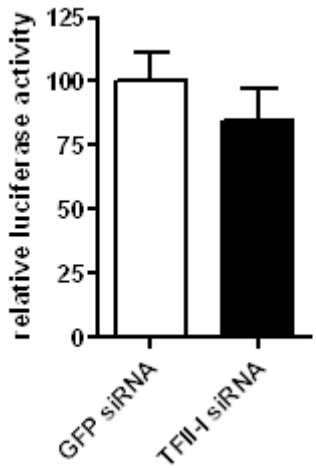


Figure 17. Knockdown of TFII-I by RNAi decreased RPL7a promoter activity.

GFP and TFII-I siRNA was firstly transfected into A549 cells. RPL7a (-90) construct was transfected after 24h post-transfection. Luciferase activity was measured at 24h post-transfection of promoter construct.

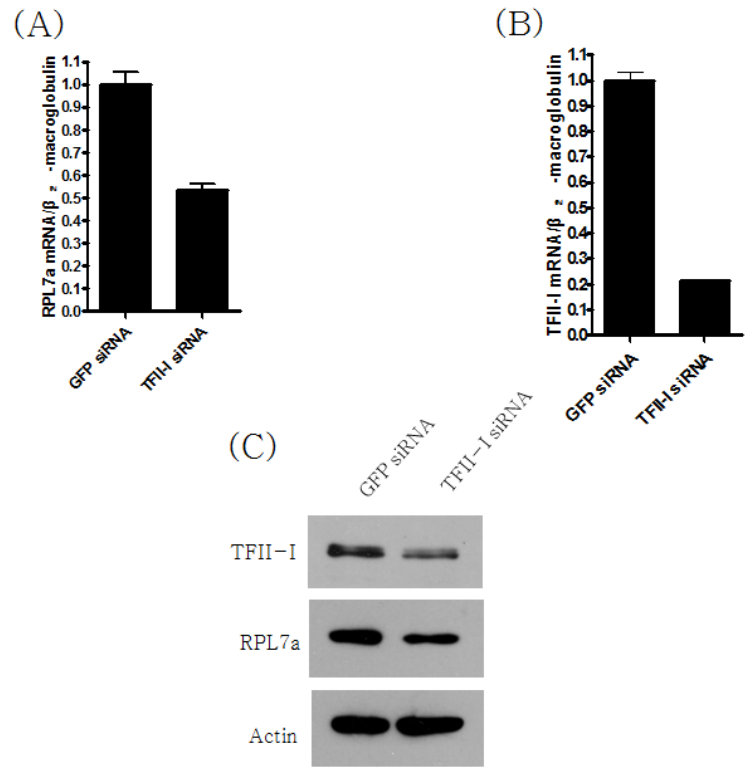


Figure 18. Knockdown of TFII-I by RNAi decreased RPL7a mRNA and protein level

TFII-I siRNA was transfected into A549 cells. After 24h, cells were harvested. mRNA level was quantified by real-time PCR (A, B). Protein level was detected by western blot (C).

(A, B) Knockdown of TFII-I by RNAi decreased RPL7a mRNA level.

(C) Knockdown of TFII-I by RNAi decreased RPL7a protein level.

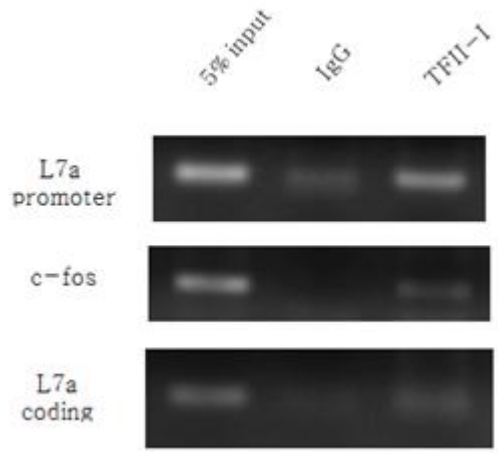


Figure 19. TFII-I directly binds to RPL7a promoter region.

TFII-I binding over promoter region of RPL7a was deduced from ChIP as described in Material and Method. The c-fos promoter and RPL7a-coding region were selected as positive and negative control, respectively.

Discussion

In this study, we identify that TFII-I and p53 are transcription factors that regulate RPL7a transcription. By decreasing RPL7a promoter activity, mRNA and protein, p53 negatively regulates RPL7a (Fig. 9-13). On the contrary, by increasing RPL7a promoter activity, mRNA and protein, TFII-I positively regulates RPL7a (Fig. 14-19). These results indicate that TFII-I and p53 are transcription factors responsible for regulation of RPL7a expression.

In addition to the role of protein synthesis, ribosomal proteins (RP) are involved in various cellular processes such as replication, transcription, RNA processing, DNA repair and even inflammation. Previous data have showed that mutations in ribosomal protein contribute to carcinogenesis (Draptchinskaia et al. 1999; Amsterdam et al. 2004; Gazda et al. 2006; Choesmel et al. 2008; Ebert et al. 2008). RPL7a mRNA expression in osteosarcoma was markedly down-regulated compared with that from normal bone (Shui-er, 2008). RPL7a, frequently lost in chromosome of osteosarcoma, is also thought to be involved in cell growth and differentiation (Shui-er, 2008).

To examine in more detail, TFII-I binding site mutant on RPL7a promoter decreased RPL7a promoter activity. But p53 binding site mutant increased it. Through chromatin immunoprecipitation, while directly binding to RPL7a promoter, each regulated RPL7a transcription. On the basis of the result, to regulate RPL7a gene, it is

recommended that TFII-I and p53 with opposite function be regulated.

It is known that TFII-I regulates the transcription of various gene in response to signaling. TFII-I is necessary for mitogen-induced transcriptional activation of the c-fos gene. TFII-I also represses β-globin gene transcription. Similarily, p53 regulates the transcription of various genes as well. In other words, as involved in the regulation of many gene, TFII-I, p53 are major factors regulating RPL7a responsible for basic event such as protein synthesis on the basis of our results.

Previously, it was reported that RPL7a was decreased during aging and TGase 2 activity was increased in aged cells, suggesting RPL7a functioned as TGase 2 inhibitor. Thus, in association with many age-related diseases such as Alzheimer's disease, Huntington's disease and cataract in TGase 2 pathologies, it is important to understand regulation mechanism of RPL7a.

In summary, we investigated transcription factor responsible for regulation of RPL7a transcription, and we identify TFII-I and p53 as major transcriptional factors. The present findings suggest the important role of TFII-I and p53 in development of RPL7a-associated diseases.

Reference

Ahvazi, B., K. M. Boeshans, et al. (2004). "Structural basis for the coordinated regulation of transglutaminase 3 by guanine nucleotides and calcium/magnesium." J Biol Chem **279**(8): 7180-7192.

Akimov, S. S. and A. M. Belkin (2001). "Cell surface tissue transglutaminase is involved in adhesion and migration of monocytic cells on fibronectin." Blood **98**(5): 1567-1576.

Akimov, S. S., D. Krylov, et al. (2000). "Tissue transglutaminase is an integrin-binding adhesion coreceptor for fibronectin." J Cell Biol **148**(4): 825-838.

Anantharaman, A., I. J. Lin, et al. (2011). "Role of helix-loop-helix proteins during differentiation of erythroid cells." Mol Cell Biol **31**(7): 1332-1343.

Antonyak, M. A., A. M. Miller, et al. (2004). "Augmentation of tissue transglutaminase expression and activation by epidermal growth factor inhibit doxorubicin-induced apoptosis in human breast cancer cells." J Biol Chem **279**(40): 41461-41467.

Balklava, Z., E. Verderio, et al. (2002). "Analysis of tissue transglutaminase function in the migration of Swiss 3T3 fibroblasts: the active-state conformation of the enzyme does not affect cell motility but is important for its secretion." J Biol Chem **277**(19): 16567-16575.

Begg, G. E., L. Carrington, et al. (2006). "Mechanism of allosteric regulation of transglutaminase 2 by GTP." Proc Natl Acad Sci U S A **103**(52): 19683-19688.

Budhram-Mahadeo, V., P. J. Morris, et al. (1999). "p53 suppresses the activation of the Bcl-2 promoter by the Brn-3a POU family transcription factor." J Biol Chem **274**(21): 15237-15244.

Burris, T. P., Z. Nawaz, et al. (1995). "A nuclear hormone receptor-associated protein that inhibits transactivation by the thyroid hormone and retinoic acid receptors." Proc Natl Acad Sci U S A **92**(21): 9525-9529.

Casadio, R., E. Polverini, et al. (1999). "The structural basis for the regulation of tissue transglutaminase by calcium ions." Eur J Biochem **262**(3): 672-679.

Cheriyath, V., Z. P. Desgranges, et al. (2002). "c-Src-dependent transcriptional activation of TFII-I." J Biol Chem **277**(25): 22798-22805.

Crusselle-Davis, V. J., K. F. Vieira, et al. (2006). "Antagonistic regulation of beta-globin gene expression by helix-loop-helix proteins USF and TFII-I." Mol Cell Biol **26**(18): 6832-6843.

Datta, S., M. A. Antonyak, et al. (2006). "Importance of Ca(2+)-dependent transamidation activity in the protection afforded by tissue transglutaminase against doxorubicin-induced apoptosis." Biochemistry **45**(44): 13163-13174.

De Falco, S., G. Russo, et al. (1993). "Human L7a ribosomal protein: sequence, structural organization, and expression of a functional gene." Gene **126**(2): 227-235.

De Laurenzi, V. and G. Melino (2001). "Gene disruption of tissue transglutaminase." Mol Cell Biol **21**(1): 148-155.

Doi-Katayama, Y., F. Hayashi, et al. (2007). "Solution structure of the general transcription factor 2I domain in mouse TFII-I protein." Protein Sci **16**(8): 1788-1792.

Ebert, B. L., J. Pretz, et al. (2008). "Identification of RPS14 as a 5q- syndrome gene by RNA interference screen." Nature **451**(7176): 335-339.

Esposito, C. and I. Caputo (2005). "Mammalian transglutaminases. Identification of substrates as a key to physiological function and physiopathological relevance." FEBS J **272**(3): 615-631.

Farmer, G., J. Colgan, et al. (1996). "Functional interaction between p53, the TATA-binding protein (TBP), andTBP-associated factors in vivo." Mol Cell Biol **16**(8): 4295-4304.

Feng, J. F., M. Readon, et al. (1999). "Calreticulin down-regulates both GTP binding and transglutaminase activities of transglutaminase Ⅱ." Biochemistry **38**(33): 10743-10749.

Fesus, L., P. J. Davies, et al. (1991). "Apoptosis: molecular mechanisms in programmed cell death." Eur J Cell Biol **56**(2): 170-177.

Fesus, L., A. Horvath, et al. (1983). "Interaction between tissue transglutaminase and phospholipid vesicles." FEBS Lett **155**(1): 1-5.

Fesus, L. and M. Piacentini (2002). "Transglutaminase 2: an enigmatic enzyme with diverse functions." Trends Biochem Sci **27**(10): 534-539.

Fesus, L. and Z. Szondy (2005). "Transglutaminase 2 in the balance of cell death and survival." FEBS Lett **579**(15): 3297-3302.

Fleckenstein, B., O. Molberg, et al. (2002). "Gliadin T cell epitope selection by tissue transglutaminase in celiac disease. Role of enzyme specificity and pH influence on the transamidation versus deamidation process." J Biol Chem **277**(37): 34109-34116.

Gaudry, C. A., E. Verderio, et al. (1999). "Cell surface localization of tissue transglutaminase is dependent on a fibronectin-binding site in its N-terminal beta-sandwich domain." J Biol Chem **274**(43): 30707-30714.

Griffin, M., R. Casadio, et al. (2002). "Transglutaminases: nature's biological glues." Biochem J **368**(Pt 2): 377-396.

Grueneberg, D. A., R. W. Henry, et al. (1997). "A multifunctional DNA-binding protein that promotes the formation of serum response factor/homeodomain complexes: identity to TFII-I." Genes Dev **11**(19): 2482-2493.

Hakimi, R. (2003). "[Kirlian photography, holistic blood diagnosis, capillarity tests. Cancer diagnosis of a different kind]." MMW Fortschr Med **145**(23): 49-50.

Hakre, S., M. I. Tussie-Luna, et al. (2006). "Opposing functions of TFII-I spliced isoforms in growth factor-induced gene expression." Mol Cell **24**(2): 301-308.

Hang, J., E. A. Zemskov, et al. (2005). "Identification of a novel recognition sequence for fibronectin within the NH2-terminal beta-sandwich domain of tissue transglutaminase." J Biol Chem **280**(25): 23675-23683.

Harms, K. L. and X. Chen (2007). "Histone deacetylase 2 modulates p53 transcriptional activities through regulation of p53-DNA binding activity." Cancer Res **67**(7): 3145-3152.

Hasegawa, G., M. Suwa, et al. (2003). "A novel function of tissue-type transglutaminase: protein disulphide isomerase." Biochem J **373**(Pt 3): 793-803.

Herman, J. F., L. S. Mangala, et al. (2006). "Implications of increased tissue transglutaminase (TG2) expression in drug-resistant breast cancer (MCF-7) cells." Oncogene **25**(21): 3049-3058.

Hinsley, T. A., P. Cunliffe, et al. (2004). "Comparison of TFII-I gene family members deleted in Williams-Beuren syndrome." Protein Sci **13**(10): 2588-2599.

Huxley, C. and M. Fried (1990). "The mouse rpL7a gene is typical of other ribosomal protein genes in it's 5' region but differs in being located in a tight cluster of CpG-rich islands." Nucleic Acids Res **18**(18): 5353-5357.

Hwang, K. C., C. D. Gray, et al. (1995). "Interaction site of GTP binding Gh (transglutaminase II) with phospholipase C." J Biol Chem **270**(45): 27058-27062.

Ientile, R., D. Caccamo, et al. (2007). "Tissue transglutaminase and the stress response." Amino Acids **33**(2): 385-394.

Innocente, S. A. and J. M. Lee (2005). "p53 is a NF-Y- and p21-independent, Sp1-dependent repressor of cyclin B1 transcription." FEBS Lett **579**(5): 1001-1007.

Jackson, T. A., H. E. Taylor, et al. (2005). "Vascular endothelial growth factor receptor-2: counter-regulation by the transcription factors, TFII-I and TFII-IRD1." J Biol Chem **280**(33): 29856-29863.

Jang, T. H., J. H. Park, et al. (2011). "Crystallization and preliminary X-ray crystallographic studies of the N-terminal domain of human ribosomal protein L7a (RPL7a)." Acta Crystallogr Sect F Struct Biol Cryst Commun **67**(Pt 4): 510-512.

Janiak, A., E. A. Zemskov, et al. (2006). "Cell surface transglutaminase promotes RhoA activation via integrin clustering and suppression of the Src-p190RhoGAP signaling pathway." Mol Biol Cell **17**(4): 1606-1619.

Kanaya, T., S. Kyo, et al. (2000). "Adenoviral expression of p53 represses telomerase activity through down-regulation of human telomerase reverse transcriptase transcription." Clin Cancer Res **6**(4): 1239-1247.

Kim, D. W., V. Cheriyath, et al. (1998). "TFII-I enhances activation of the c-fos promoter through interactions with upstream elements." Mol Cell Biol **18**(6): 3310-3320.

Kim, J. H., H. E. Choy, et al. (2001). "Transglutaminase-mediated crosslinking of specific core histone subunits and cellular senescence." Ann N Y Acad Sci **928**: 65-70.

Kotsakis, P. and M. Griffin (2007). "Tissue transglutaminase in tumour progression: friend or foe?" Amino Acids **33**(2): 373-384.

Kroes, R. A., A. Jastrow, et al. (2000). "The identification of novel therapeutic targets for the treatment of malignant brain tumors." Cancer Lett **156**(2):

191-198.

Kubicka, S., F. Kuhnel, et al. (1999). "p53 represses CAAT enhancer-binding protein (C/EBP)-dependent transcription of the albumin gene. A molecular mechanism involved in viral liver infection with implications for hepatocarcinogenesis." J Biol Chem **274**(45): 32137-32144.

Lai, T. S., A. Hausladen, et al. (2001). "Calcium regulates S-nitrosylation, denitrosylation, and activity of tissue transglutaminase." Biochemistry **40**(16): 4904-4910.

Lee, K. C., A. J. Crowe, et al. (1999). "p53-mediated repression of alpha-fetoprotein gene expression by specific DNA binding." Mol Cell Biol **19**(2): 1279-1288.

Lesort, M., K. Attanavanich, et al. (1998). "Distinct nuclear localization and activity of tissue transglutaminase." J Biol Chem **273**(20): 11991-11994.

Liu, S., R. A. Cerione, et al. (2002). "Structural basis for the guanine nucleotide-binding activity of tissue transglutaminase and its regulation of transamidation activity." Proc Natl Acad Sci U S A **99**(5): 2743-2747.

Lohr, K., C. Moritz, et al. (2003). "p21/CDKN1A mediates negative regulation of transcription by p53." J Biol Chem **278**(35): 32507-32516.

Lorand, L. and R. M. Graham (2003). "Transglutaminases: crosslinking enzymes with pleiotropic functions." Nat Rev Mol Cell Biol **4**(2): 140-156.

Lu, S. and P. J. Davies (1997). "Regulation of the expression of the tissue transglutaminase gene by DNA methylation." Proc Natl Acad Sci U S A **94**(9): 4692-4697.

Lucena, J., S. Pezzi, et al. (2010). "Essential role of the N-terminal region of TFII-I in viability and behavior." BMC Med Genet **11**: 61.

Mager, W. H. (1988). "Control of ribosomal protein gene expression." Biochim Biophys Acta **949**(1): 1-15.

Maguire, B. A. and R. A. Zimmermann (2001). "The ribosome in focus." Cell **104**(6): 813-816.

Mammoto, A., K. M. Connor, et al. (2009). "A mechanosensitive transcriptional mechanism that controls angiogenesis." Nature **457**(7233): 1103-1108.

Mastroberardino, P. G., M. G. Farrace, et al. (2006). ""Tissue" transglutaminase contributes to the formation of disulphide bridges in proteins of mitochondrial respiratory complexes." Biochim Biophys Acta **1757**(9-10):

1357-1365.

Mhaouty-Kodja, S. (2004). "Ghalpha/tissue transglutaminase 2: an emerging G protein in signal transduction." Biol Cell **96**(5): 363-367.

Milakovic, T., J. Tucholski, et al. (2004). "Intracellular localization and activity state of tissue transglutaminase differentially impacts cell death." J Biol Chem **279**(10): 8715-8722.

Mishra, S., G. Melino, et al. (2007). "Transglutaminase 2 kinase activity facilitates protein kinase A-induced phosphorylation of retinoblastoma protein." J Biol Chem **282**(25): 18108-18115.

Mishra, S. and L. J. Murphy (2004). "Tissue transglutaminase has intrinsic kinase activity: identification of transglutaminase 2 as an insulin-like growth factor-binding protein-3 kinase." J Biol Chem **279**(23): 23863-23868.

Mishra, S. and L. J. Murphy (2006). "The p53 oncoprotein is a substrate for tissue transglutaminase kinase activity." Biochem Biophys Res Commun **339**(2): 726-730.

Mishra, S., A. Saleh, et al. (2006). "Phosphorylation of histones by tissue transglutaminase." J Biol Chem **281**(9): 5532-5538.

Murphy, M., J. Ahn, et al. (1999). "Transcriptional repression by wild-type p53 utilizes histone deacetylases, mediated by interaction with mSin3a." Genes Dev **13**(19): 2490-2501.

Murthy, S. N., J. H. Wilson, et al. (1998). "Cross-linking sites of the human tau protein, probed by reactions with human transglutaminase." J Neurochem **71**(6): 2607-2614.

Murzin, A. G., S. E. Brenner, et al. (1995). "SCOP: a structural classification of proteins database for the investigation of sequences and structures." J Mol Biol **247**(4): 536-540.

Nakaoka, H., D. M. Perez, et al. (1994). "Gh: a GTP-binding protein with transglutaminase activity and receptor signaling function." Science **264**(5165): 1593-1596.

Nunes, I., P. E. Gleizes, et al. (1997). "Latent transforming growth factor-beta binding protein domains involved in activation and transglutaminase-dependent cross-linking of latent transforming growth factor-beta." J Cell Biol **136**(5): 1151-1163.

Nurminskaya, M. and M. T. Kaartinen (2006). "Transglutaminases in mineralized

tissues." Front Biosci **11**: 1591-1606.

Ohlsson, C., N. Kley, et al. (1998). "p53 regulates insulin-like growth factor-I (IGF-I) receptor expression and IGF-I-induced tyrosine phosphorylation in an osteosarcoma cell line: interaction between p53 and Sp1." Endocrinology **139**(3): 1101-1107.

Ori, A., A. Zauberman, et al. (1998). "p53 binds and represses the HBV enhancer: an adjacent enhancer element can reverse the transcription effect of p53." EMBO J **17**(2): 544-553.

Pedersen, L. C., V. C. Yee, et al. (1994). "Transglutaminase factor XIII uses proteinase-like catalytic triad to crosslink macromolecules." Protein Sci **3**(7): 1131-1135.

Piacentini, M., M. G. Farrace, et al. (2002). "Transglutaminase overexpression sensitizes neuronal cell lines to apoptosis by increasing mitochondrial membrane potential and cellular oxidative stress." J Neurochem **81**(5): 1061-1072.

Pinkas, D. M., P. Strop, et al. (2007). "Transglutaminase 2 undergoes a large conformational change upon activation." PLoS Biol **5**(12): e327.

Piper, J. L., G. M. Gray, et al. (2002). "High selectivity of human tissue transglutaminase for immunoactive gliadin peptides: implications for celiac sprue." Biochemistry **41**(1): 386-393.

Piredda, L., M. G. Farrace, et al. (1999). "Identification of 'tissue' transglutaminase binding proteins in neural cells committed to apoptosis." FASEB J **13**(2): 355-364.

Riley, T., E. Sontag, et al. (2008). "Transcriptional control of human p53-regulated genes." Nat Rev Mol Cell Biol **9**(5): 402-412.

Roy, A. L. (2001). "Biochemistry and biology of the inducible multifunctional transcription factor TFII-I." Gene **274**(1-2): 1-13.

Roy, A. L. (2007). "Signal-induced functions of the transcription factor TFII-I." Biochim Biophys Acta **1769**(11-12): 613-621.

Roy, A. L., H. Du, et al. (1997). "Cloning of an inr- and E-box-binding protein, TFII-I, that interacts physically and functionally with USF1." EMBO J **16**(23): 7091-7104.

Roy, A. L., M. Meisterernst, et al. (1991). "Cooperative interaction of an initiator-binding transcription initiation factor and the helix-loop-helix activator

USF." Nature **354**(6350): 245-248.

Seto, E., A. Usheva, et al. (1992). "Wild-type p53 binds to the TATA-binding protein and represses transcription." Proc Natl Acad Sci U S A **89**(24): 12028-12032.

Siegel, M. and C. Khosla (2007). "Transglutaminase 2 inhibitors and their therapeutic role in disease states." Pharmacol Ther **115**(2): 232-245.

Singh, U. S., M. T. Kunar, et al. (2001). "Role of transglutaminase Ⅱ in retinoic acid-induced activation of RhoA-associated kinase-2." EMBO J **20**(10): 2413-2423.

Sun, Y., X. R. Zeng, et al. (2004). "P53 down-regulates matrix metalloproteinase-1 by targeting the communications between AP-1 and the basal transcription complex." J Cell Biochem **92**(2): 258-269.

Thomazy, V. and L. Fesus (1989). "Differential expression of tissue transglutaminase in human cells. An immunohistochemical study." Cell Tissue Res **255**(1): 215-224.

Thut, C. J., J. L. Chen, et al. (1995). "p53 transcriptional activation mediated by coactivators TAFII40 and TAFII60." Science **267**(5194): 100-104.

Truant, R., H. Xiao, et al. (1993). "Direct interaction between the transcriptional activation domain of human p53 and the TATA box-binding protein." J Biol Chem **268**(4): 2284-2287.

Tussie-Luna, M. I., D. Bayarsaihan, et al. (2002). "Physical and functional interactions of histone deacetylase 3 with TFII-I family proteins and PIASxbeta." Proc Natl Acad Sci U S A **99**(20): 12807-12812.

Vaarala, M. H., K. S. Porvari, et al. (1998). "Several genes encoding ribosomal proteins are over-expressed in prostate-cancer cell lines: confirmation of L7a and L37 over-expression in prostate-cancer tissue samples." Int J Cancer **78**(1): 27-32.

Verderio, E. A., T. Johnson, et al. (2004). "Tissue transglutaminase in normal and abnormal wound healing: review article." Amino Acids **26**(4): 387-404.

Vullhorst, D. and A. Buonanno (2005). "Multiple GTF2I-like repeats of general transcription factor 3 exhibit DNA binding properties. Evidence for a common origin as a sequence-specific DNA interaction module." J Biol Chem **280**(36): 31722-31731.

Warner, J. R. and C. R. Nierras (1998). "Trapping human ribosomal protein

genes." Genome Res **8**(5): 419-421.

Wen, Y. D., W. D. Cress, et al. (2003). "Histone deacetylase 3 binds to and regulates the multifunctional transcription factor TFII-I." J Biol Chem **278**(3): 1841-1847.

Wool, I. G. (1996). "Extraribosomal functions of ribosomal proteins." Trends Biochem Sci **21**(5): 164-165.

Wu, Y. and C. Patterson (1999). "The human KDR/flk-1 gene contains a functional initiator element that is bound and transactivated by TFII-I." J Biol Chem **274**(5): 3207-3214.

Yamamoto, T. (2000). "Molecular mechanism of monocyte predominant infiltration in chronic inflammation: mediation by a novel monocyte chemotactic factor, S19 ribosomal protein dimer." Pathol Int **50**(11): 863-871.

Zhang, J., M. Lesort, et al. (1998). "Modulation of the in situ activity of tissue transglutaminase by calcium and GTP." J Biol Chem **273**(4): 2288-2295.

Zhang, Y., J. S. Wang, et al. (2004). "Repression of hsp90beta gene by p53 in UV irradiation-induced apoptosis of Jurkat cells." J Biol Chem **279**(41): 42545-42551.

Zhu, Y., H. Lin, et al. (2001). "Modulation of expression of ribosomal protein L7a (rpL7a) by ethanol in human breast cancer cells." Breast Cancer Res Treat **69**(1): 29-38.

국문초록

리보좀 단백질은 세포질에서 단백질 합성을 촉매하는 리보좀의 주요 구성성분이다. 리보좀 단백질은 단백질을 합성하는 기능 외에도 복제, 전사, RNA 가공, DNA 복제 심지어 염증과 같은 다양한 세포 과정을 수행한다. 트랜스글루타미네이즈 2 (TGase 2)는 단백질을 수식하는 효소로서, 다양한 스트레스에 의해 활성화되어, 섬유화, 신경퇴행성 질환, 암과 같은 다양한 질환의 발생과 관련되어 있다. 선행연구를 통해 세포 내 TGase 2 의 활성이 리보좀 단백질 L7a (RPL7a)에 의해 억제됨을 알게 되었다. 따라서 RPL7a 의 발현을 조절하는 전사인자를 확인하고자 하였다. Luciferase 활성 측정을 통하여 RPL7a 프로모터의 활성부위를 확인하였으며, p53 와 TFII-I 를 작용 가능성이 있는 전사인자로 확인하였다. p53 의 과발현 시, RPL7a 의 프로모터 활성 및 mRNA, 단백질 발현 수준이 감소하였다. 반대로 TFII-I 를 RNAi 방법을 통해 발현 감소시킨 결과 RPL7a 의 프로모터 활성 및 mRNA, 단백질 발현 수준이 감소하였다. 또한 p53 과 TFII-I 가 RPL7a 프로모터 부위에 직접적으로 결합함을 확인하였다. 이러한 결과는 RPL7a 의 발현이 p53 과 TFII-I 에 의해 서로 상반되게 조절되는 것을 나타내며, p53 과 TFII-I 에 의한 RPL7a 의 발현 조절이 TGase 2 의 활성과 관련된 여러 질환의 발생과정에 작용할 가능성을 시사한다.

주 요 어: 리보좀 단백질 L7a, TFII-I, p53, 전사조절

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