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# Expression of Yippee-Like 5 (YPEL5) Gene During Activation of Human Peripheral T Lymphocytes by Immobilized Anti-CD3

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Yippee-like proteins, which have been identified as the homolog of *Drosophila* yippee protein containing a zinc-finger domain, are known to be highly conserved among eukaryotes. However, their functional roles are still poorly understood. Recently we initiated ordered differential display (ODD)-polymerase chain reaction (PCR) to isolate genes of which expressions are altered following activation of human T cells. On the ODD-PCR image, one PCR-product detected in unstimulated T cells was not detectable at the time when the activated T cells traversed near $G_1$/S boundary following activation by immobilized anti-CD3. Cloning and nucleotide sequence analysis revealed that the PCR-product was yippee-like 5 (YPEL5) gene, which was known as a human homolog of the *Drosophila* yippee gene. Northern blot analysis confirmed the amount of ~2.2 kb YPEL5 mRNA expression detectable in unstimulated T cells was sustained until 1.5 hr after activation and then rapidly declined to undetectable level by 5 hr. Ectopic expression of YPEL5 gene in human cervix epitheloid carcinoma HeLa cells caused a significant reduction in cell proliferation to the level of 47% of the control. Expression of GFP-YPEL5 fusion protein in HeLa cells showed its nuclear localization. These results demonstrated that the expression level of human YPEL5 mRNA was negatively regulated in the early stage of T cell activation, and suggested that YPEL5 might exert an inhibitory effect on the cell proliferation as a nuclear protein.

**Key words** : ODD-PCR, yippee-like 5 gene, T cell activation, cell cycle

## Introduction

Mature T lymphocytes, which were developed in thymus, migrate to the peripheral lymphoid organs and tissues and remain in a resting ($G_0$) state. The interaction of antigen or mitogenic lectin with specific receptors on $G_0$ T cells initiates a cascade of biochemical events to induce the expression of a wide variety of genes essential for activation and proliferation, and subsequent immune functions [5,6,21]. The IL-2 and IL-2R are thought to be among the more critical genes for the proliferation of activated T cells, in that binding of IL-2 to IL-2R expressed on the surface of activated T cells induces the signal necessary for the $G_1$/S transition [14-16,20].

Recently we have initiated an ordered differential display (ODD)-PCR, a method for displaying 3'-end *Rsa*I-restriction fragments of cDNA [3,13], to isolate the genes which are changed during polyclonal activation of human peripheral T lymphocytes by immobilized anti-CD3. It is likely that this identification of genes differentially expressed during the activation of T cells is an important step towards understanding the molecular mechanism that regulates the activation and proliferation of T cells. The 366 cDNA fragments exhibiting a distinctive increase or decrease in the expression level in the ODD-PCR image were cloned and analyzed for the nucleotide sequence. One 262-bp cDNA clone that appeared to be significantly down-regulated in T cells following activation by immobilized anti-CD3 activation was the human yippee-like 5 (YPEL5) gene, which is known to have homology with *Drosphila* yippee gene. Yippee protein has been initially isolated by the yeast two-hybrid method as a *Drosophila* protein containing a zinc-binding domain, which interacts with a member of the immunoglobulin superfamily, haemolin, whose expression level is significantly upregulated upon bacterial infection [17]. It has been reported that there are 100 YPEL genes from 68 species including animals, plants, and fungi [7]. Comparison of these YPEL genes revealed a high similarity at amino acid sequence

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levels and a consensus sequence of $CX_2CX_{19}GX_3LX_5NX_{13}GX_8CX_2CX_4GWXYX_{10}KX_6E$ in all the YPEL family proteins. In humans, five yippee-like genes (YPEL1 through YPEL5) have been found with high homology (43.8~96.6%) at amino acid sequence levels. However, little has been known about their functional roles. In the present studies, we have investigated the expression of YPEL5 in human peripheral T lymphocytes following activation by immobilized anti-CD3. In addition, the effect of ectopic expression of YPEL5 on the growth of human cervix epitheloid carcinoma HeLa cells was investigated to examine the functional role of YPEL5. The results showed that the YPEL5 mRNA was easily detectable in the resting ($G_0$) T cells but became undetectable in 5 hr after activation. Additional results showed that the ectopic expression of YPEL5 in HeLa cells resulted in a significant reduction in the cell proliferation, suggesting its suppressive role in the cell growth.

## Materials and Methods

### Kits, oligo primers, enzymes, reagents, media and cells

The SuperScrip™ system for cDNA synthesis was purchased from Life Technologies (Gaithesburg, MD, USA). All restriction enzymes and DNA modifying enzymes including T4 DNA ligase, RNase, and T4 polynucleotide kinase were purchased from Boehringer Mannheim (Indianapolis, IN, USA). A DNA sequencing kit (Omnibase™), Taq DNA polymerase, and pGEM-T Easy Vector System I were purchased from Promega (Madison, WI, USA). ExpressHyb™ hybridization solution for Northern blot analysis was from Clontech (Palo Alto, CA, USA). TaqStart™ antibody for PCR amplification, Radioactive materials including [α-$^{32}$P]dCTP (~3,000 Ci/mmol), [γ-$^{32}$P]ATP (~3,000 Ci/mmol), and random primer labeling kit were from Amersham (Arlington Heights, IL, USA). [$^3$H]TdR (2 Ci/mmol) and nylon membrane (GeneScreen Plus™) were from NEN Biotechnology System (Boston, MA, USA). A Geneclean II kit was obtained from Bio 101 (Vista, CA, USA). The host strain used for cDNA cloning was *Escherichia coli* JM 109. All components of bacterial media were from Difco Laboratories (Detroit, MI, USA). HeLa cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FBS (Hyclone, Utah, USA), and 100 μg/ml gentamycin. Rabbit antiserum raised against recombinant human YPEL5 protein that was expressed using *Escherichia coli* system was prepared essentially as described [8].

### Isolation and activation of human peripheral T cells

To prepare human peripheral T cells, heparinized blood obtained by vein puncture from healthy laboratory personnel was centrifuged at 800 g for 20 min over Histopaque-1077 (Sigma, St Louis, MO, USA). T cells were isolated from mononuclear cells with a T-cell enrichment column kit (R&D Systems, Minneapolis, MN, USA) as described by the manufacturer. For activation, T cells were treated with monoclonal anti-CD3 at 4°C for 1 hr and then layered on culture plates previously coated with rabbit anti-mouse IgG antibody. The incorporation of [$^3$H] thymidine (TdR) into DNA by $1.5 \times 10^5$ T cells activated in each well of a 96-well plate for 2 hr at the indicated times was determined as previously described [10].

### Ordered differential display (ODD)-polymerase chain reaction (PCR)

ODD-PCR was performed essentially as reported previously [3,13]. After total RNA from human peripheral T cells treated by immobilized anti-CD3 for 26 hr or 30 hr was extracted, double-stranded cDNA was synthesized with the T-primer 5′-GCGAGTCGACCG$(T)_{13}$ using the SuperScript™ System. The synthesized cDNA samples were digested by *Rsa*I, and half were then used for ligation with a pseudo-double-stranded adaptor (a long oligo, 5′-GCGTGAAGACGACAGAAAGGGCG-TGGTGCGGAGG GCGGT; a short oligo, 5′-ACCGCCCTCCGC). Ligation was performed overnight at 16°C in a 10 μl volume with 2 μM adaptor. Then 1 μl of a 1:5 dilution of the ligation mixture was used for PCR with an adaptor-specific primer (5′-TGTAGCGTGAAGACGACAGAA) and the T-primer. Amplification was carried out in a 20 μl volume containing 1X reaction buffer (5 mM KCl, 10 mM Tris-HCl pH 9.0, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 15 μM ammonium sulfate), with 250 μM dNTPs, 0.3 μM primers and 2.5 units of Taq DNA polymerase mixed with TaqStart™ antibody. Polymerase was added to the PCR mixtures at 72°C and incubated for 10 min before the first denaturation stage. The amplification profile included 20 cycles of 95°C for 30 sec, 65°C for 30 sec, and 72°C for 1.5 min. One microliter of a 1:20 dilution (~1 ng/μl) of this PCR product in water served as the initial material for the amplification of the

simplified 3'-end cDNA subsets. For the amplification, individual AdE-primers (Adaptor specific Extended; 5'-AGG GCGTGGTGCGGAGGGCGGTCCNN, where NN is GC or AG) were $^{32}$P-labelled by T4 polynucleotide kinase (Boehringer Mannheim, Indianapolis, IN, USA) according to the manufacturer's instructions. The reaction was conducted for 30 min at 37°C and stopped by heating the tube for 1 min at 100°C. Then 2 μl of this mixture was added to 8 μl of PCR mixture containing 5 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM $MgCl_2$, 15 μM ammonium sulfate, 250 μM dNTPs, 2.5 U of Taq DNA polymerase mixed with TaqStart™ antibody, 0.2 μM non-labeled TE-primer (T-Extended; 5'-GCGAGTCGACCG$(T)_{13}$ NN, where NN is AG, GG, GA, GT or GC), and 1 ng of the representative 3'-end cDNA fragment sample under investigation. PCR was performed with the following conditions: 23 cycles of 95°C for 30 s, 69°C for 30 s, and 72°C for 1.5 min. To resolve PCR products, 2 μl of each reaction were electrophoresed on 6% polyacrylamide sequencing gel. Detection of the amplified cDNA fragments was visualized by autoradiography after the gel was dried and exposed to X-ray film at -70°C. Differentially displayed cDNAs on the dried sequencing gel were eluted into 20 μl of TE buffer (pH 8.0) at 70°C for 2 hr. Two microliters of the eluant were reamplified with T-primer 5'-GCGAGTCG ACCG$(T)_{13}$ and nonextended adaptor-specific primer (5'-A GGGCGTGGTGCGGAGGGCGGT) for 20 cycles. A reamplified DNA fragment was electrophoresed on 2% agarose gel, purified using the Geneclean II kit, and then used for cloning and sequence analysis.

### DNA sequence analysis and homology search

The cDNA fragment differentially expressed was cloned using a pGEM-T Easy Vector System I (Promega, Madison, WI, USA) and was sequenced using the Omnibase™ DNA cycle sequencing system (Promega) according to the manufacturer's instructions. The sequence information of the cDNA fragment was compared with GenBank and European Molecular Biology Laboratory (EMBL) databases using the BLAST search program of the National Center for Biotechnology Information (NCBI), National Institutes of Health (NIH), Bethesda, MD.

### Cell lysate, protein quantitation, and Western blot analysis

The cells were suspended in lysis buffer (20 mM Tris, 137 mM NaCl, 1 mM $Na_3VO_4$, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 2.5 μg/ml E64, 1% Nonidet P-40, pH 8.0), disrupted by sonication, and extracted at 4°C for 30 min. After centrifugation at 14,000 rpm for 20 min, the supernatant was obtained as cell lysate. Protein quantitation was performed using a Micro BCA kit (PIERCE, Rockford, IL, USA). Twenty micrograms of cell lysates were subjected to electrophoresis on a 4-12% NuPAGE gradient gel (Invitrogen Corporation/Novex, Carlsbad, CA, USA) and electrotransferred to Immobilon-P membrane. The membrane was allowed to react with individual primary antibodies and then with horse radish peroxidase conjugated with Donkey anti-rabbit IgG antibody. Detection of each protein was visualized using an ECL Western blotting detection system according to the manufacturer's instructions.

### Flow cytometry analysis

Approximately, $1 \times 10^6$ T cells were fixed with 67% cold ethanol for 1 hr. The cells were washed with PBS, and resuspended with 12.5 μg RNase in 250 μl of 1.12% sodium citrate buffer (pH 8.45). Incubation was continued at 37°C for 30 min before staining of the cellular DNA with 250 μl of propidium iodide, at 50 μg/ml, for 20 min. The stained cells were analyzed on a FACScan flow cytometer for relative DNA content, based on increased red fluorescence [11].

### Northern blot analysis

Total RNA was extracted and isolated by solubilization in guanidine thiocyanate as described elsewhere [11]. Equivalent amounts of total RNA were electrophoresed on 1% formaldehyde-agarose gels and transferred to GeneScreen Plus membranes. The nylon membrane was hybridized overnight at 62°C with cDNA probe radiolabeled with [α-$^{32}$P]dCTP by random primer labeling method [4], and washed at 65°C.

### Transfection of HeLa cells and fluorescence microscopy

HeLa cells were transfected with individual plasmid DNA constructs using Transfectene kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The proliferation of HeLa cells transfected with pCAGGS empty vector or pCAGGS-YPEL5 construct was analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay reflecting cell viability, as described

elsewhere [9]. To observe localization of GFP and GFP-YPEL5 under fluorescence microscope, HeLa cells transfected with either GFP vector or GFP-YPEL5 construct were fixed with 4% paraformaldehyde for 30 min. Images were collected at × 200 using Zeiss Axioplan 2 imaging Fluorescence Microscope (Carl Zeiss, Gottingen, Germany).

### Statistical analysis

Unless otherwise indicated, each result in this paper is representative of at least three separate experiments. Values represent the mean ± standard deviation (SD) of these experiments. Statistical significance was calculated with Student's *t*-test. *P* values less than 0.05 were considered significant.

## Results and Discussion

### Identification of the human YPEL5 cDNA among the differentially expressed transcripts during polyclonal activation of human T cells by immobilized anti-CD3

We have employed the ordered differential display (ODD)-polymerase chain reaction (PCR) method to investigate the expression profiles of human T lymphocytes during immobilized anti-CD3 activation to identify novel, previously uncharacterized genes. Since the model of polyclonal activation of human peripheral T cells in vitro is known to provide a population of resting ($G_0$) T cells that, following activation, synchronously reenter the cell cycle and traverse $G_1$ phase to enter S phase, this cell model is useful for determining the requirement of gene expression regulations for resting cells to enter the cell cycle, which can be induced in T cells after antigen recognition [11,12]. Whereas the expression of the majority of essential genes is either not detectable or is at basal level in $G_0$ T cells until activation through cell surface receptors to enter the cell cycle, there may be the other group of essential genes whose expression is at the highest level in the $G_0$ state and needs to be down-regulated for the activation of the $G_0$ T cells. In this context, we have performed the ODD-PCR using total RNA extracted from human peripheral T cells and human peripheral T cells activated by immobilized anti-CD3 for 26 hr or 30 hr. A representative example of ODD-PCR for five 3′ cDNA fragment subsets compared in three samples are shown in Fig. 1. Many bands representing PCR-amplified 3′-end *Rsa*I-restriction fragments of cDNAs exhibited different patterns in abundance, suggesting

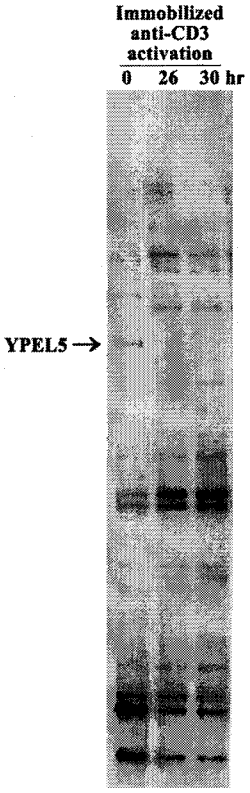


Fig. 1. ODD-PCR images for different 3′ cDNA fragments compared in three samples. Total RNAs from unstimulated human peripheral T cells, and the T cells activated by immobilized anti-CD3 for 26 hr or 30 hr were reverse transcribed, and sequentially the obtained 3′-end *Rsa*I-restriction fragments of cDNAs were amplified by PCR as described in Materials and Methods. The PCR products were electrophoresed on 6% polyacrylamide sequencing gel, and detection of the amplified cDNA fragments was visualized by autoradiography after the gel was dried and exposed to X-ray film.

that their corresponding mRNA might be differentially expressed in human T cells after immobilized anti-CD3 activation. Various cDNA fragments exhibiting a distinctive increase or decrease in the expression level upon the ODD-PCR image were eluted from the gel, cloned, and analyzed for their nucleotide sequences. The sequence for each cDNA fragment was compared with those in the GenBank database using the BLAST search program. One of 366 cDNA clones that appeared to be significantly upregulated or down-regulated upon immobilized anti-CD3 activation of human T cells was 262-bp in size and showed 100% similarity with the 3′-end of the human YPEL5 gene (GenBank AB098739), which was identified as the human homolog of *Drosphila* yippee gene [7,17]. The human

YPEL5 gene has a predicted 121 amino acid open reading frame, encoding a 14 kDa protein. As shown in Fig. 2, the sequence similarity of human YPEL5 was 100% identical to the murine and rat counterparts at amino acid sequence levels, and showed 70%, 39%, 39%, and 31% identity with individual yippee-like proteins from *Mus musculus* (GenBank accession no. P62700), *Rattus norvegicus* (GenBank accession no. NP_001030298), *Drosphila melanogaster* (GenBank accession no. Q9XZF0), *Arabidopsis thaliana* (GenBank accession no. CAB38286), *Caenorhabditis elegans* (GenBank accession no. O44440), and *Saccharomyces cerevisiae* (GenBank accession no. P38191). It has been reported that there are 100 YPEL genes from 68 species including animals, plants, and fungi [7]. Comparison of these YPEL genes revealed a high similarity at amino acid sequence levels and a consensus sequence of $CX_2CX_{19}GX_3LX_5NX_{13}GX_8CX_2CX_4GWXY$ $X_{10}$ $KX_6E$ in all the YPEL family proteins. *Drosophila* yippee protein was initially isolated by the yeast two-hybrid method as a protein that could physically interact with haemolin, whose expression level was highly upregulated upon bacterial infection [17].

## Expression of YPEL5 mRNA during activation of human T cells by immobilized anti-CD3

It is generally accepted that the level of transcript represented by an ODD-PCR image sometimes does not reflect the real expression pattern of the gene [3,13]. To examine whether the expression level of YPEL5 detected in the ODD-PCR image reflects the real expression pattern of YPEL5 mRNA altered along with immobilized anti-CD3 activation of human T cells, the expression of YPEL5 mRNA was investigated by Northern blot analysis in human T cells during the activation. When human peripheral T cells were activated by immobilized anti-CD3, the cells appeared to begin the incorporation of [$^3$H] thymidine (TdR) into DNA in 28 hr after activation, demonstrating that the $G_0$ T cells reentered the cell cycle and started the S phase for DNA synthesis in 28 hr after activation (Fig. 3A). Under these conditions, the expression of the YPEL5-specific mRNA (~2.2 kb) was easily detectable in unstimulated human peripheral T cells, and its expression level appeared to sustain until 1.5 hr after activation (Fig. 3B). However, the YPEL5 mRNA was not detected 5 hr after activation. On the other hand, the expression of 3-phosphoglycerate dehydrogenase (PHGDH) that catalyses the transition of 3-phosphoglycerate into 3-phosphohydroxy

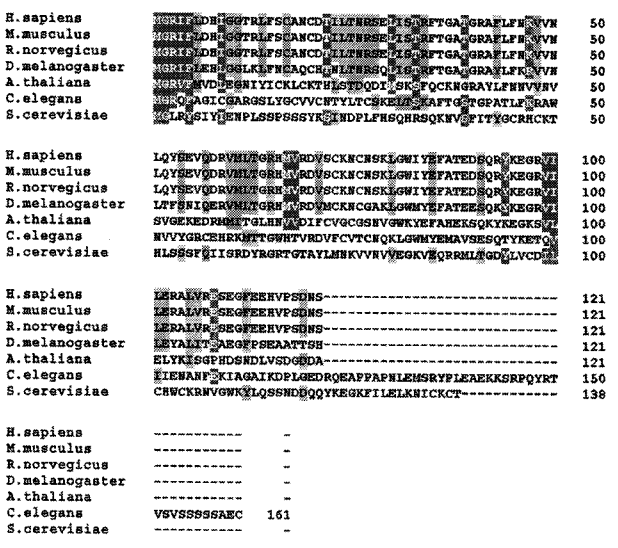


Fig. 2. Comparison of amino acid sequences of YPEL5 from *Homo sapiens, Mus musculus, Rattus norvegicus, Drosphila melanogaster, Arabidopsis thaliana, Caenorhabditis elegans,* and *Saccharomyces cerevisiae.* Amino acids are displayed in single-letter abbreviation after alignment for maximal identity using the CLUSTAL W program.

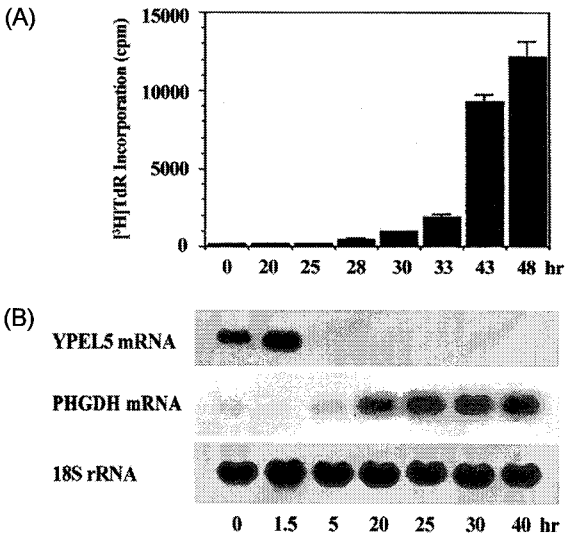


Fig. 3. Kinetic analysis of the [$^3$H]TdR-incorporation (A), and the alteration in the level of YPEL5 mRNA and PHGDH mRNA (B) during activation of human peripheral T cells by immobilized anti-CD3. Human peripheral T cells were activated by immobilized anti-CD3. For [$^3$H]TdR-incorporation assay, murine $G_0$ T cells ($10^5$/well) were activated by immobilized anti-CD3 in a 96-well plate, and pulsed for 2 hr with 1 μCi of [$^3$H]TdR at times indicated. Each value is expressed as mean ± SD (n = 3). For Northern analysis, total RNA (10 μg) from each time point were electrophoresed, transferred, and probed with $^{32}$P-labeled YPEL5, PHGDH, and 18S rRNA cDNA.

pyruvate, the first and rate-limiting step in the phosphorylated pathway of L-serine biosynthesis [1,18,19], was also analyzed. Since the expression of PHGDH was known to accumulate in the S phase and support cellular replication [1,2], we decided to investigate the expression level of PHGDH mRNA in the activated T cells as a T-cell activation marker. There was no detectable PHGDH mRNA until 5 hr, at which time the PHGDH mRNA was detected as a faint band. However, the level of PHGDH mRNA increased and reached a maximum in 25 hr when the activated T cells were about to incorporate [$^3$H]TdR. These results demonstrated that the human peripheral T cells in the $G_0$ phase were able to begin the cell cycle progression following activation by immobilized anti-CD3, and entered the S phase in 28 hr. These results also demonstrated that, in accordance with the result of ODD-PCR, the expression level of YPEL5 mRNA was rapidly down-regulated long before the T cells enter the S phase after activation by immobilized anti-CD3.

### Ectopic expression of the human YPEL5 in HeLa cells

In order to understand further the functional role of the human YPEL5, we have examined the effect of ectopic expression of YPEL5 in human cervix epitheloid carcinoma HeLa cells. A previous analysis by indirect immunefluorescence staining has indicated that human YPEL5 protein is localized at nucleus in COS-7 cells [7]. To investigate the subcellular localization of YPEL5 in HeLa cells, HeLa cells grown on cover slip were transfected with GFP-YPEL5 cDNA construct for 48 hr, and the localization of GFP-YPEL5 fusion protein was compared with that of GFP control protein. As shown in Fig. 4A and 4B, the GFP protein appeared to be localized at both nuclear and cytoplasmic regions, whereas the GFP-YPEL5 fusion protein was localized at nucleus. These results confirmed the nuclear localization of YPEL5, which was previously observed by indirect fluorescence immunostaining in COS-7 cells. In addition, HeLa cells grown on cover slips were transfected with either pCAGGS empty vector or pCAGGS-YPEL5 construct to investigate the effect of ectopic expression of YPEL5 protein on HeLa cells. After transfection of HeLa cells for 48 hr, the cells transfected with pCAGGS empty vector or pCAGGS-YPEL5 construct were harvested and their proliferation potentials were compared by MTT assay. As shown in Fig. 5A, although

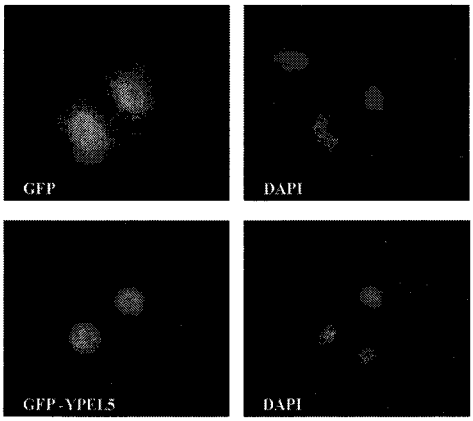


Fig. 4. Western analysis of ectopic expression of human YPEL5 protein (A) in HeLa cells, and the effect of YPEL5 expression on the proliferation of HeLa cells (B). HeLa cells untreated, and transfected with pCAGGS vector or pCAGGS-YPEL5 construct were harvested 48 hr after transfection, and their cell lysates were subjected to Western blot analysis. For proliferation assay, equivalent HeLa cells ($2 \times 10^4$/well), which were harvested 48 hr after transfection, were added to 96-well plate and incubated for additional 48 hr, and the final 4 hr was incubated with MTT. The cells were sequentially processed to assess the colored formazan crystal produced from MTT as an index of cell viability.

the proliferation of HeLa cells transfected with pCAGGS empty vector was essentially the same as that of HeLa cells untreated, the proliferation of HeLa cells transfected with pCAGGS-YPEL5 construct was declined and showed only 47% of the control. Under the same conditions, the ectopic expression of YPEL5 protein in HeLa cells was easily detectable by Western blot analysis using rabbit polyclonal anti-human YPEL5 antibody. These results suggested that a functional role of human YPEL5 protein might be associated with suppression of the cell proliferation.

In summary, this report demonstrates that YPEL5 mRNA expression which is detectable in the human peripheral T cells in the resting ($G_0$) stage can be rapidly down-regulated upon activation by immobilized anti-CD3, and suggests that the ectopic expression of YPEL5 may confer a suppressive role to the proliferation of HeLa cells.

## Acknowledgment

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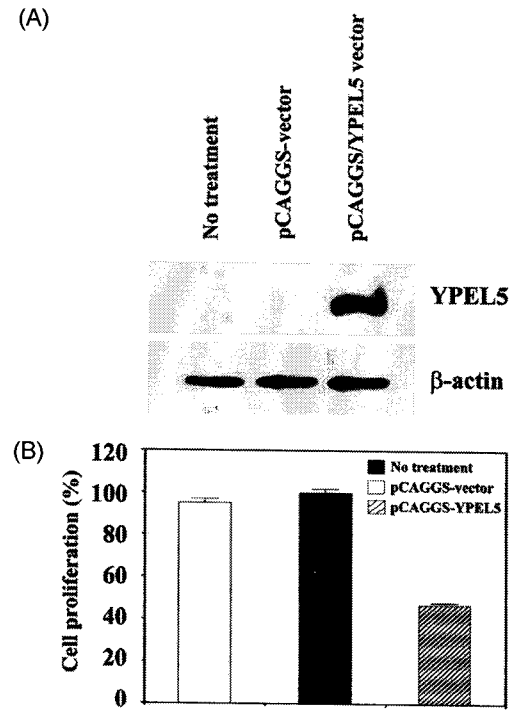


Fig. 5. Subcellular localization of GFP and GFP-YPEL5 in HeLa cells. To observe localization of GFP and GFP-YPEL5 fusion protein under fluorescence microscope, HeLa cells transfected with either GFP empty vector or GFP-YPEL5 construct for 48 hr were fixed with 4% paraformaldehyde for 30 min. The cells were then washed five times with with PBS containing 0.2% Tween 20, and stained with 100 $\mu$M DAPI solution. Images were collected at x200 using Zeiss Axioplan 2 imaging Fluorescence Microscope.

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## 초록 : 인체 말초혈액의 활성화 과정 중 yippee-like 5 (YPEL5) 유전자의 발현 양상

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Yippee 패밀리를 구성하는 yippee-유사 단백질들은 한 개의 zinc-finger 도메인을 지닌 *Drosophila* yippee 단백질의 homolog로서 모든 진핵생물에 존재하는 것으로 알려졌으나 그 기능은 밝혀진 바가 없다. 인체 T 림프구의 활성화과정 중 발현수준이 변화하는 유전자들을 선별하기 위해 인체 말초혈액에서 분리한 resting T 세포, immobilized anti-CD3에 의해 26시간 혹은 30시간 동안 활성화시킨 T 세포로부터 각각 정제한 total RNA를 이용하여 ODD-PCR을 수행한 결과, resting T 세포에서는 발현되지만 immobilized anti-CD3 활성화에 의해 세포주기를 개시하여 $G_1$/S boundary에 도달한 T 세포들로부터는 전혀 발현되지 않는 흥미로운 유전자로서 *Drosophila* yippee 단백질 유전자의 인체 homolog인 YPEL5 유전자를 분리하였다. 노던 블로팅법으로 T 세포 활성화에 뒤이은 YPEL5 mRNA의 발현 변화를 조사한 결과, ~2.2 kb 크기의 YPEL5 mRNA는 resting T 세포를 비롯하여 immobilize anti-CD3에 의한 활성화 후 1.5시간 까지는 확인되었으나 활성화 후 5시간 이후부터 48시간에 이르는 시간에는 전혀 확인되지 않았다. YPEL5 단백질을 GFP-fusion 단백질로서 인체 암세포주인 HeLa 세포에 transfection하여 발현시킨 결과, GFP-YPEL5 단백질이 모두 핵에 위치하는 것으로 나타나 YPEL5 단백질이 핵단백질임을 확인하였다. 또한 YPEL5의 기능을 규명하기 위해 YPEL5 발현벡터를 HeLa 세포에 transfection 하고 발현시켜 HeLa 세포의 증식에 미치는 YPEL5의 영향을 MTT assay로 분석한 결과, vector plasmid를 transfection시킨 대조구의 47% 수준으로 세포증식이 감소하는 것으로 나타났다. 이러한 결과들은 YPEL5 mRNA의 발현이 T 세포 수용체를 통한 T 세포 활성화의 초기단계에 현저히 감소됨을 보여주며, 또한 YPEL5가 핵단백질로서 세포증식에 대해 저해효과를 미칠 수 있음을 시사한다.