Korean J Biol Sci 6: 335-339, 2002

Overexpression of SRG3/SWI3 Protein Disrupts the Cell Cycle Progression in Mature T Cells and Yeast

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Key Words:
SRG3
SWI3
Polyploidy
Cell cycle
T cell
Yeast

Mouse T cells overexpressing the SRG3 protein displayed morphological changes; the cells were enlarged and their shapes were irregular compared to the normal parental cells. In addition, growth rate of the cells was dramatically reduced and their DNA contents were increased. The increased DNA contents were due to an increase in number of chromosomes in these cells. We have observed similar results in *S. cerevisiae* cells overexpressing the yeast SWI3 protein. Yeast cells overexpressing SWI3 protein displayed the phenotypes of highly elongated buds and 2C DNA content. These results suggest that the SRG3/SWI3 protein plays an important role in cell growth and cell cycle progression.

The SWI/SNF complex facilitates transcriptional activation by antagonizing the repressive actions of chromatin (Kruger and Herskowitz, 1991; Hirschhorn et al., 1992; Peterson and Tamkun, 1995). The subunit proteins of SWI/SNF complex were initially identified in *Saccharomyces cerevisiae* (*S. cerevisiae*) as a positive regulator of HO, a gene involved in mating type switching (Stern et al., 1984; Sternberg et al., 1987), and SUC2, a glucose-repressible gene that encodes the enzyme invertase (Carlson et al., 1981; Neigeborn and Carlson, 1984). These SWI gene products were subsequently found to be required for the transcriptional activation of many other genes (Estruch and Carlson, 1990; Laurent et al., 1990; Happel et al., 1991; Cote et al., 1994). Such activities of SWI/SNF proteins are closely interconnected and they seem to function as components of a complex that associates with gene-specific activators (Laurent et al., 1991; Peterson and Herskowitz, 1992; Cairns et al., 1994; Peterson et al., 1994).

Previously, we have isolated a new mouse gene, the SWI3-related gene (SRG3) which encodes the protein homologous to both yeast SWI3 and human BAF155 proteins of SWI/SNF complex (Jeon et al., 1997). The SRG3 protein is highly expressed in immature thymocytes, and down regulated after positive selection during T cell differentiation (Choi et al., 2001). The transcriptional activity of SRG3 was enhanced by interaction with glucocorticoid receptor (GR) (Han et al., 2001; Kim et al., 2001), suggesting that SRG3 has crucial roles in glucocorticoid (GC) sensitivity, differentiation and proliferation of thymocytes.

Suppressors of *swi* and *snf* mutations include mutations in the genes encoding histones, suggesting that SWI/SNF proteins oppose repression by nucleosomes (Hirschhorn et al., 1992; Winston and Carlson, 1992; Kruger et al., 1995). In addition, *swi/snf* mutants are defective in the remodeling of chromatin at the SUC2 promoter (Hirschhorn et al., 1992). A novel 15-subunit complex with the capacity to remodel the structure of chromatin (RSC) has been isolated on the basis of homology to the SWI/SNF complex in *S. cerevisiae* (Cairns et al., 1996). These RSC subunits are significantly similar to but more abundant than SWI/SNF complex and are essential for mitotic growth.

Recently, it was reported that overexpression of BRG1, a core component of SWI/SNF complex, induces growth arrest of SW13 cell line (Shanahan et al., 1999). Another report demonstrated that BRG1 and BAF155 are inactivated by phosphorylation during mitosis (Sif et al., 1998). Therefore, it was proposed that components of SWI/SNF should be inactivated by phosphorylation during mitosis. However, except for BRG1, the role of other components of SWI/SNF complex on cell cycle has not been reported.

In this paper, we examined the effect of SRG3 protein in EL4, a murine T cell line. Overexpression of SRG3 in EL4 cells resulted in growth retardation and polyploidy. Interestingly, yeast cells overexpressing SWI3 showed similar phenotypes such as retarded growth rate and elongated multi buds.

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Materials and Methods

Cells and plasmids

The yeast strain CY165 (*MATa, swi3Δ::trp1-Δ1, HO-lacZ, ura3-52, leu2-Δ1, his3-Δ200, ade2-101, lys-801*) cells and yCP50 plasmid containing the SWI3 gene were generous gifts of Dr. Peterson (Univ. of Massachusetts, Worcester). As a control, DBY747 (*MATa, his3, leu2-3, leu2-112, ura3-52, trp1-289*) cell was used. Yeast cells were grown in synthetic minimal medium (0.67% Bacto-yeast nitrogen base without amino acids) (GibcoBRL) supplemented with leucine, histidine, adenine, and lysine to a mid-log phase. The murine thymoma cell line S49.1 and the lymphoma cell line EL4 were purchased from American Tissue Culture Collection and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Hyclone). The plasmid pLXSN-SRG3 contains the 4.7 kb of BamHI fragment of SRG3 gene under the control of LTR promoter. Stable EL4 cells (EL4-SRG3) were established by selecting G418 resistant cells after electroporation, and maintained in appropriated medium supplemented with 1 μg/mL G418.

10^4 cells of selected or parental cells were diluted in culture media. Optical density (O.D.) was measured at wavelength 600 nm by spectrophotometry (UnicamII).

Immunoblot analysis

EL4 and S49.1 cells were harvested, sonicated and pelleted by centrifugation. The supernatant was boiled in SDS-PAGE loading buffer. For immunoblot analysis, the proteins separated on SDS-PAGE were electro-transferred to nitrocellulose paper, and incubated in blocking solution (3% non-fat milk, 50 mM Tris-Cl, pH 7.5, and 150 mM NaCl) with gentle agitation for 2 h. After the blot was incubated with anti-SRG3 antiserum, the specific band was detected by treating the blot with anti-rabbit IgG conjugated with alkaline phosphatase in blocking solution (100 mM Tris-Cl, pH 9.5, 100 mM NaCl, and 10 mM $MgCl_2$) containing 165 μg/mL BCIP and 330 μg/mL NBT.

Flow cytometry

Cell cycle progression of T and yeast cells was measured by flow cytometry as described (Nicoletti et al., 1991). Briefly, the harvested cells were fixed with 70% ethanol and stained with 50 μg/mL of propidium iodide (PI) and 10 μg/mL RNAse A for 30 min at 37℃. After washing with PBS buffer, the stained cells were analyzed using the FACStar^{plus} and Cell Quest program (Becton Dickinson) for their DNA content.

Karyotyping and microscopy

EL4 and EL4-SRG3 cells were treated with 1 μg colcemid (Sigma) for 3 h. The cells were harvested and suspended in prewarmed hypotonic solution (50 mM KCl) and incubated for 10 min at 37℃. After fixation, the cells were dropped on slide glasses and stained with 1% Giemsa solution. The stained chromosomes were observed under a microscope at 1000× magnification. The EL4 and yeast cells were fixed with 4% paraformaldehyde and their morphology was observed under a microscope.

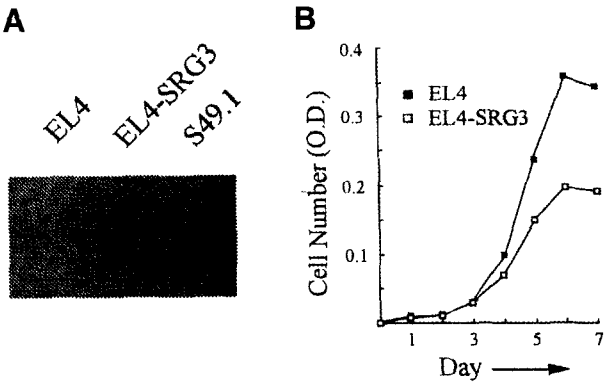

Fig. 1. Effect of overexpressed SRG3 protein in mature T cell line, EL4. A, EL4 and EL4-SRG3 cell lysates were immunoblotted with anti-SRG3 antiserum. Expression of SRG3 protein was increased by 4-5 folds compared to that of EL4, but still was lower than that in S49.1, immature thymoma. B, EL4-SRG3 cells have reduced growth rate.

Results

Overexpression of SRG3 protein in a mouse T cell line Overexpression of BRG1, a core component of SWI/SNF complex, induced growth arrest of the SW13 cell line derived from a tumor of adrenal cortex (Shanahan et al., 1999). Another report demonstrated that BRG1 and BAF155 were phosphorylated during mitosis, the phosphorylated BRG1 and BAF155 bound to cyclin E, and the activity of SWI/SNF complex was suppressed during mitosis (Sif et al., 1998). These results suggested that components of SWI/SNF, at least BRG1 and BAF155, should be inactivated by phosphorylation during mitosis.

Based on these results, we investigated the effect of SRG3 protein in mature T cells when it is highly and abnormally expressed. The DNA construct expressing the *Srg3* gene was transfected into EL4 (EL4-SRG3). Expression level of the SRG3 protein in EL4-SRG3 was confirmed by immunoblot analysis (Fig. 1A). It was higher, by 4-5 folds, than in EL4 parental cell line, but still not as high as that in the S49.1 immature thymocyte cell line.

EL4-SRG3 cells showed a reduced growth rate compared to EL4 cells and more rapidly reached the plateau in growth curve (Fig. 1B). In addition, EL4-SRG3 cells displayed morphological changes such as much larger cell and nucleus, irregular cell shape, and incomplete cell division (Fig. 2). As shown in Fig. 3, EL4-SRG3 cells showed increased DNA contents and sensitivity to glucocorticoids as reported previously

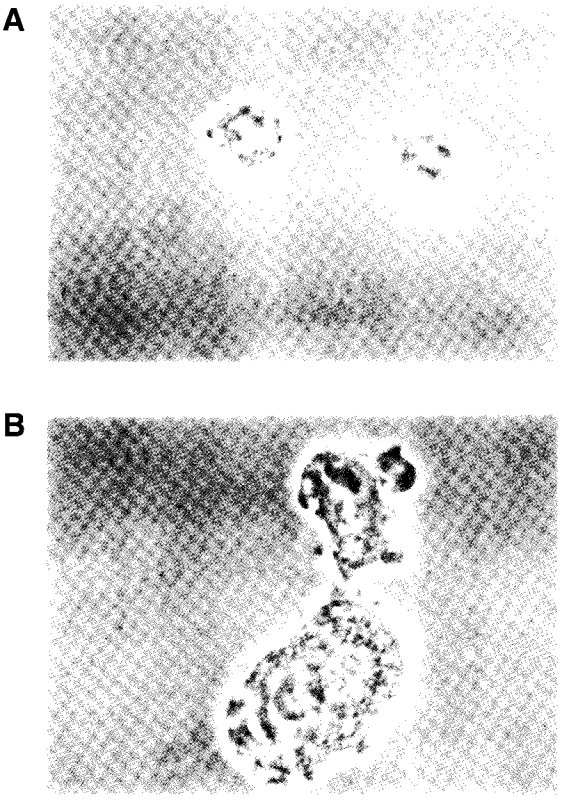

Fig. 2. Overexpression of SRG3 protein induced morphological changes. EL4 (A) and EL4-SRG3 (B) cells were fixed with 4% paraformaldehyde and observed by microscopy. The cells overexpressing SRG3 protein show irregular cell shape and large nucleus.

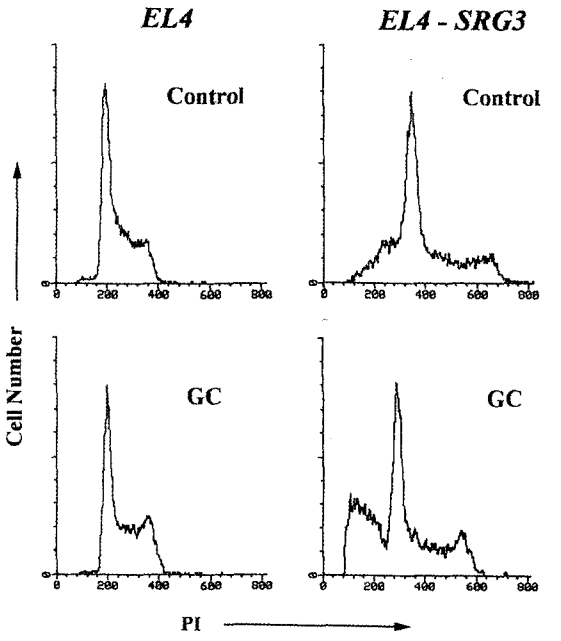

Fig. 3. Cells overexpressing SRG3 protein showed increased DNA contents and increased GC-induced apoptosis. EL4 and EL4-SRG3 cells were treated with ethanol or GC for 72 h, and then stained with 50 μg/mL PI. EL4-SRG3 cells show increased DNA contents (right panel) and increased GC-mediated apoptosis. The subdiploid peak (right, bottom) represents apoptotic cell population.

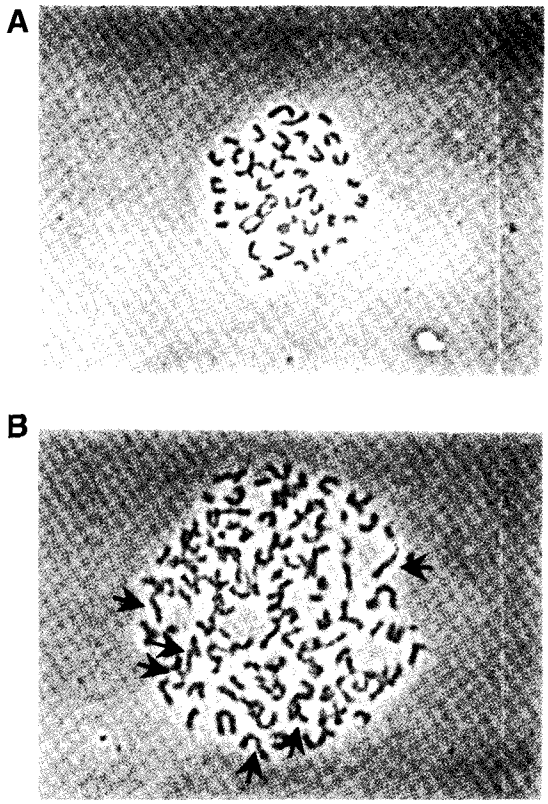

Fig. 4. Chromosome abnormality was induced by overexpressed SRG3 protein. EL4 (A) and EL4-SRG3 (B) cells were treated with colcernid and stained with 1% Giemsa solution. Chromosome number in EL4 and EL4-SRG3 were 40 and 110-120, respectively. In EL4-SRG3 cells, some chromosomes show asymmetric shape in their long arms (arrow).

(Jeon et al., 1997).

Because EL4-SRG3 had larger nucleus, it was suggested that the increase in DNA contents might be due to an increase in chromosome number. To examine this possibility, karyotypes of EL4 and EL4-SRG3 were determined. Karyotyping analysis showed that most EL4-SRG3 cells were in polyploidy (Fig. 4). When EL4 cells were examined for chromosome number by karyotyping, most cells had 40 chromosomes. However, most EL4-SRG3 contained 110-120 chromosomes per single cell. In addition, some chromosomes in EL4-SRG3 cells showed an asymmetrical difference in the size of both arms, although most of the chromosomes in EL4 cell showed a symmetric shape (Fig. 4). It is not clear why these changes were induced, but we suggest that the SRG3 protein may play an important role during regulation of cell cycle progression.

Overexpression of SWI3 protein in S. cerevisiae

BRG1 and SRG3 are components of SWI/SNF complex and involved in cell cycle progression. In yeast, when expression of the yeast *Snf2*-related gene *Sth1/Nps1* was repressed, cell growth was arrested and re-replication of DNA occurred without passage through

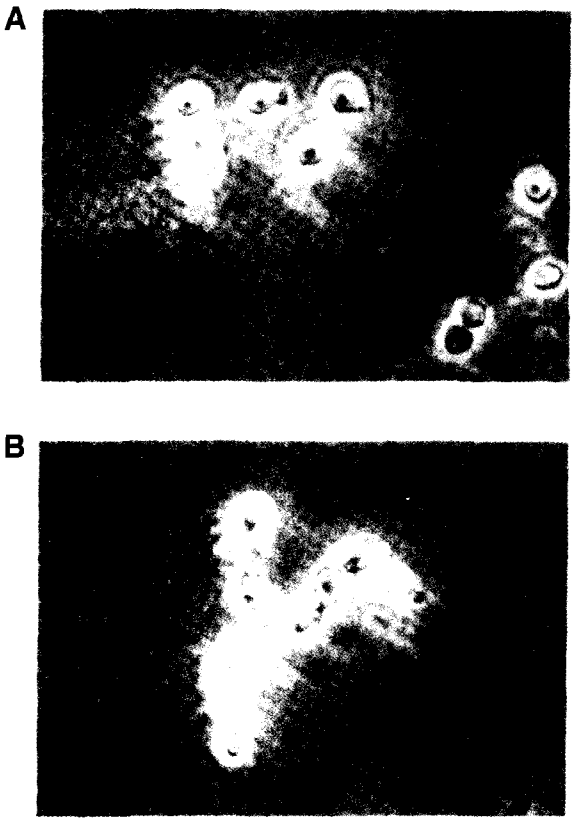

Fig. 5. Overexpression of SWI3 protein in *S. cerevisiae* resulted in multi bud phenotype. The yCP50 vector containing the *Swi3* gene was transformed in the DBY747 cells. Overexpression of SWI3 protein was confirmed by immunoblotting with anti-SWI3 antiserum. The cells harboring overexpressed SWI3 protein showed multi-bud and incomplete cell division (B) compared to parental cells, DBY747 (A).

mitosis (Tsuchiya et al., 1992). Therefore, it was interesting whether yeast SWI3 plays similar roles in cell cycle as SRG3.

Overexpression of SWI3 in *S. cerevisiae* induced similar phenomena as shown in EL4-SRG3 cells. The yeast DBY747 cells transformed with yCP50 vector containing the *Swi3* gene underwent incomplete cell division and formed multi-buds (Fig. 5). The overexpression also displayed a reduced growth rate and an increased DNA content compared to its parental cells (Fig. 6). It seems that the SWI3 protein also plays an important role in yeast cell growth and cell cycle progression.

Discussion

When SRG3 was overexpressed in EL4 cells (EL4-SRG3), the cells showed reduced growth rate and abnormal DNA content. Overexpression of SWI3 protein in *S. cerevisiae* also induced similar phenomena. The morphological and cytogenetic changes of these cells strongly suggest that the SRG3 protein may also be involved in cell growth and cell cycle progression.

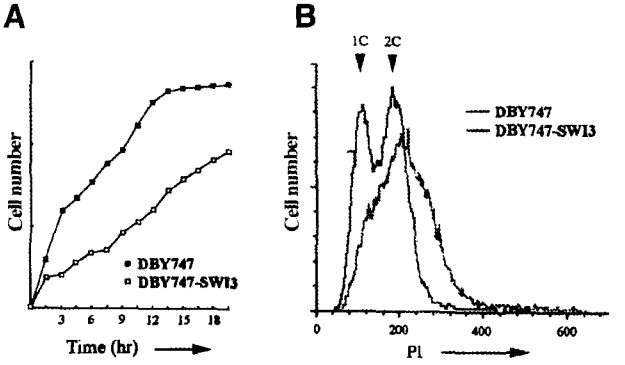

Fig. 6. Cells overexpressing SWI3 protein showed retarded growth rate and increased DNA contents. A, Growth rate of cells containing overexpressed SRG3 protein was significantly reduced. B, DNA contents of wild type and the cells containing the overexpressed SWI3 protein.

The yeast SNF2-related gene *Sth1/Nps1* is a novel CDC gene involved in G2 phase control. When the expression of Sth1/Nps1 protein was repressed, cell growth was arrested and re-replication of DNA occurred without passage through mitosis (Tsuchiya et al., 1992). The SNF5 homolog of SWI/SNF complex Sfh1 interacts functionally and physically with Sth1/Nps1, in a novel nucleosome-restructuring complex called RSC for "remodels the structure of chromatin" (Tsuchiya et al., 1992; Cao et al., 1997). Sfh1 is essential for viability and also required for progression through G2/M as Sth1 is. Recently, it was reported that Sth1 is associated with the SWI3 homolog Swh3 and that they are functionally distinct from the SWI/SNF complex (Treich and Carlson, 1997). The components of RSC complex are essential for cell survival and have critical roles in regulation of cell cycle progression (Tsuchiya et al., 1992; Cao et al., 1997). The phenotype of cells containing overexpressed SWI3/SRG3 was similar to RSC-deficient cells. In fact, EL4-SRG3 cells showed significant reduction in p27kip and cdk2 expression (data not shown), which are important in cell cycle progression (Kwon et al, 1997; Sheaff et al., 1997). However, the exact role of SRG3 protein in cell cycle control is yet to be determined.

Acknowledgements

We thank Dr. C. Peterson for providing yCP50. This work was supported by IVI-affiliated Laboratory Research Collaboration Grant to RHS.

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[Received October 7, 2002; accepted November 5, 2002]